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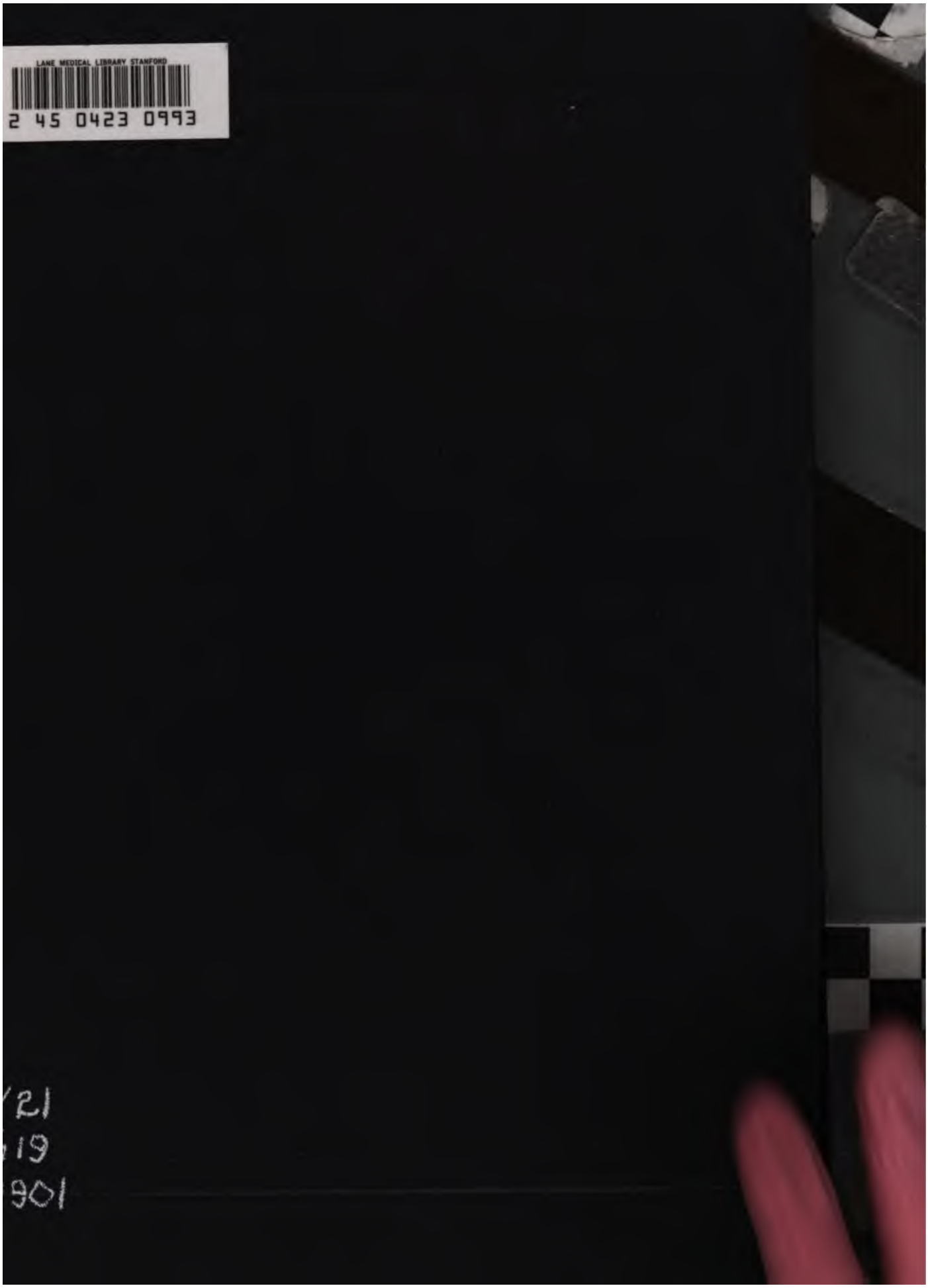

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN No. 2.

B. T. GALLOWAY, Chief of Bureau.

SPERMATOGENESIS AND FECUNDATION OF ZAMIA.

BY

HERBERT J. WEBBER,

PHYSIOLOGIST, VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS,
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ISSUED DECEMBER 28, 1901.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF THE CHIEF,
Washington, D. C., August 1, 1901.

SIR: I have the honor to transmit herewith the manuscript of a paper entitled Spermatogenesis and Fecundation of Zamia, by Dr. Herbert J. Webber, Physiologist, in Charge of the Plant Breeding Laboratory, Vegetable Pathological and Physiological Investigations, this Bureau. I respectfully recommend its publication as Bulletin No. 2 of the Bureau series.

Respectfully,

B. T. GALLOWAY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

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PREFACE.

The following technical paper on the Spermatogenesis and Fecundation of Zamia, by Dr. Herbert J. Webber, embodies the results of investigations started by him several years ago at our tropical laboratory in Florida. The time at his disposal for this work was very limited, so that it has extended over a much longer period than was at first expected.

As an aid to the practical work of plant breeding it is highly important that a more thorough knowledge of the reproduction of plants be gained. Such investigations throw light on the phenomena of heredity, which are at the foundation of plant breeding work. The present paper is of especial interest because the large size of the sexual nuclei in Zamia has enabled Dr. Webber to work out some of the phenomena of fecundation with greater exactness than has ever been done before.

ALBERT F. WOODS.

OFFICE OF THE PATHOLOGIST AND PHYSIOLOGIST,
Washington, D. C., July 20, 1901.

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SPERMATOGENESIS AND FECUNDATION OF ZAMIA.

By HERBERT J. WEBBER,
Physiologist.

INTRODUCTION.

Within recent years renewed interest has been awakened in the phenomena accompanying spermatogenesis in plants, due largely to researches on certain cycadaceous plants and Pteridophytes in which the cilia of the spermatozoid have been found to develop from a body resembling a centrosome. This interest was greatly enhanced by the fact that the enormous spermatozoids of the Cycadaceæ and *Ginkgo* were but newly discovered, and in groups of plants where motile sexual cells had not been known to occur. Zoological activity in this direction has also been very great in recent years, a number of cases having been described in which it is claimed that the axial filament is developed directly from the centrosome which here forms the so-called middle piece (*Mittelstück*) of the spermatozoon.

The writer's investigations on the spermatogenesis and fecundation of *Zamia* and *Ginkgo* began in 1897, and since that time several preliminary papers and short notes have been issued in various places. In 1897 three preliminary papers were published in the Botanical Gazette and a short note in the Report of the British Association for the Advancement of Science. In 1898 additional observations were described in a report read at the American Association for the Advancement of Science, and in 1900 still further observations, particularly on the morphology and development of the pollen tube apparatus, were described. The present paper covers the ground connectedly, more in detail, and with illustrations. The investigation is of considerable interest as throwing light on the phenomena accompanying fecundation and on the relation of the cilia-forming organs of spermatogenous cells to centrosomes or centrospheres.

SUMMARY OF RECENT LITERATURE.

The following summary of the recent literature on the spermatogenesis of Pteridophytes, cycadaceous plants, and *Ginkgo*, arranged in order of publication, will give an idea of the advancement of our knowledge in this direction.

The occurrence of spermatozoids in *Ginkgo* was first announced by Hirase, a Japanese botanist, in a short note in Japanese in the Botanical Magazine of October, 1896, and a few months later in a preliminary contribution, "Untersuchungen über das Verhalten des Pollens von *Ginkgo biloba*," published in Botanisches Centralblatt, Nos. 2 and 3, Band 69, appearing January, 1897. The important features here described, other than the fact of the occurrence of motile spermatozoids in one of the phanerogams where they had never before been known to occur, was the structure of the mature spermatozoid, which was described as consisting of a nucleus completely surrounded by cytoplasm. While Belajeff had strongly maintained that the cytoplasm entered into the structure of the spermatozoids of certain ferns and of *Chara*, this was yet considered doubtful. Hirase says:

Die Spermatozoiden von *Ginkgo* haben eine andere Gestalt als die der höheren Kryptogamen. Sie sind eiförmig, 82μ lang bei 49μ Breite; in der Mitte sitzt der Zellkern, welcher durch Cytoplasma völlig umschlossen ist. Der Kopf besteht aus drei nie erstreckbar gebauten Spiralwindungen, worauf viele Cilien wurzeln, auch ist ein spitzer Schwanz vorhanden.

About the same time (November 20, 1896) Professor Ikeno, another Japanese botanist associated with Hirase in the University of Tokyo, announced the discovery of spermatozoids in *Cycas*. The first announcement appeared in the Botanical Magazine, November 20, 1896, and was almost immediately followed by a short statement in Botanisches Centralblatt (61). Here, as in the case of Hirase's preliminary announcements above referred to, the articles are limited to a simple statement of the occurrence of the spermatozoids and their structure, nothing being given as to their development. Ikeno wrote:

Sie sind etwas grösser als die letzteren [those of *Ginkgo*] und enthalten Zellkern und Cytoplasma. Der Zellkern nimmt den mittleren Theil derselben ein und wird von dem Cytoplasma völlig umhüllt. Der Kopf besteht aus vier Spiralwindungen und trägt sehr reichlich Cilien. Im pollinschlauch findet man zur richtigen Zeit je zwei durch die Theilung der generativen Zelle entstandene Spermatozoiden.

In the June, 1897, number of the Annals of Botany, Ikeno and Hirase (68) together published a short note in English announcing the discovery of spermatozoids in *Cycas* and *Ginkgo*. However, no important additional facts were given.

In the June, 1897, number of the Botanical Gazette the writer's first preliminary paper, entitled "Peculiar Structures Occurring in the Pollen Tube of *Zamia*," appeared. The pollen tube apparatus was described and the central cell (generative cell) was traced through its growth up to the close of its division just preceding the formation of the spermatozoids. Very large centrosome-like bodies were found in the central cell and their growth, structure, separation of outer membrane into segments during division, and disconnection with spindle formation was described and figured. The discovery of motile spermatozoids was also announced, but their development was not explained.

In the July, 1897, number of the Botanical Gazette the writer's second preliminary paper on "The Development of the Antherozoids of *Zamia*" appeared. The membrane formed from the outer wall of the centrosome-like body was found to grow into an extended band which assumed the form of a helicoid spiral, became appressed against the plasma membrane of the cell, and gave rise to the cilia of the spermatozoid. The cilia appear first as small protuberances on the band and gradually grow in length until mature. The structure of the mature spermatozoids, their motions in the pollen tube and while swimming free in sugar solutions, were described and figured. Their action in the process of fecundation was also described.

Almost simultaneously with the publication of the writer's second preliminary paper on "The Development of the Antherozoids of Zamia," Belajeff, a Russian botanist, published two preliminary papers describing the presence of a cilia-forming organ or *Nebenkern* in the spermatids of Filicineæ and Equisetineæ (8 and 9), which is doubtless identical with the similar organ in *Zamia* and *Ginkgo*. They apparently originate in the spermatids, since no trace of them could be discovered in the spermatid mother-cells in the resting condition or during karyokinesis. The first changes visible in the metamorphosis of the spermatid cells occur in these organs. They gradually become extended into a thread which assumes the form of a helicoid spiral of which the extended turns of the posterior end surround the nucleus. The cilia of the spermatozoids are developed from the anterior end of this spiral, appearing first as small protuberances on the thread, which finally become greatly extended and form the cilia.

In the October number of the Botanical Gazette the writer's third preliminary paper, "Notes on the Fecundation of Zamia and the Pollen-tube Apparatus of Ginkgo," appeared. In this paper the important features described were that in the fecundation of *Zamia* the spermatozoid enters the protoplasm at the apex of the egg cell where it undergoes disintegration, the nucleus escaping from the cytoplasm and spiral band of the spermatozoid and passing thence alone to the egg nucleus with which it unites. The fecundation is thus a union of cells, the cytoplasmic structures of the spermatozoid fusing with the cytoplasm of the egg cell, while the sperm nucleus passes on and fuses with the egg nucleus. The spiral band which is developed from the centrosome-like body was shown to have no connection with the process of fecundation, remaining, after the escape of the nucleus, intact at the apex of the archegonium and gradually disappearing during the development of the embryo. The centrosome-like bodies in the generative cell of *Ginkgo*, first described by Hirase and termed "attractive spheres" (56), were found to originate *de novo* in the cytoplasm, and their undoubted identity with the centrosome-like bodies in *Zamia* was pointed out. These bodies in *Zamia* and

Ginkgo, being distinct from typical centrosomes in their main function, namely, that of forming the motile cilia of the spermatozoid, were here named *blepharoplasts*.

Early in 1898 Ikeno, in a short paper in Flora (69), announced the occurrence in the spermatids of *Cycas revoluta* of cilia-forming organs like the blepharoplasts of *Zamia*, and claimed that these and the *Nebenkern* of the Filicineæ and Equisetineæ are nothing but genuine centrosomes.

Later in 1898 Shaw (102) described the occurrence of blepharoplasts in the spermatid mother cells of *Onoclea* and *Marsilia* which developed into a cilia-bearing band, as in the cases described by Belajeff. Shaw, however, was able to demonstrate the occurrence of similar bodies in the primordial mother cells (*Urmutterzellen*). At the close of the division which gives rise to the primordial mother cells small round bodies, called by Shaw *blepharoplastoids*, became visible. During the resting stage of the nuclei the blepharoplastoids divide into two, increase in size, and remain near the nucleus. As soon as the nuclei of these cells prepare to divide the pair of blepharoplastoids move away from the nucleus and take a position at one side in the cytoplasm about midway between the pole and the equator of the spindle until near the end of the metakinesis stage, when they disappear. The blepharoplasts proper appear as very small bodies, one at each pole of the spindle, about the time that the *blepharoplastoids* disappear, or occasionally slightly before. During the resting stage of the spermatid mother cells the blepharoplast divides into two, and these gradually separate and move to a position in the cytoplasm near where the poles of the next spindle is formed, but always slightly to one side of this. After the completion of the division they become extended into the cilia-bearing band.

In June, 1898, Hirase's complete monograph on the fecundation and embryology of *Ginkgo* appeared (62), describing in detail the development of the spermatozoids. The cilia of the spermatozoids are here developed from a membrane, which is formed from the blepharoplast, the same as in *Zamia*, differing only in minor details. The writer considers the blepharoplast to be nothing more than a centrosome and calls it such throughout his monograph. Hirase described the spermatozoids of *Ginkgo* as having a well-developed tail attached to the posterior end, which would make them seemingly quite different from the spermatozoids described by the writer. Many features of this monograph will be discussed in the present paper in comparison with *Zamia*.

In the report of the Boston meeting of the American Association for the Advancement of Science, published in Science November 11, 1898, the writer (127) described the phenomena connected with the bursting of the blepharoplast in *Zamia* at the close of the division giving rise to the spermatids and the formation of the cilia-bearing band.

The blepharoplast was described as increasing in size and separating into segments or plates, which ultimately form numerous round or elliptical granules that collect into a compact mass in the place occupied by the blepharoplast. These granules gradually fused together, forming the cilia-bearing membrane of the spermatozoid.

Slightly later in the same month, November 23, Ikeno's complete monograph on the development of the sexual organs and the process of fecundation in *Cycas revoluta* appeared (70). The details of the development of the spermatozoids, described by Ikeno for *Cycas*, are almost entirely identical with those previously described by the writer for *Zamia*, differing only in two important details—the connection of a protuberance from the nucleus with the ciliferous band during its growth and elongation and in the presence of a tail attached to the posterior end of the spermatozoid. In the fecundation of *Cycas*, as in that of *Zamia*, the ciliferous band and cytoplasm remain at the apex of the archegonium, the nucleus only fusing with the egg nucleus. Many features of this monograph will be discussed in the present paper in comparison with *Zamia*.

In December, 1898, Fujii (39 and 40), another Japanese botanist, called attention to an apparently serious contradiction between the observations of Ikeno, Hirase, and the writer as to the presence of a tail in the spermatozoids, and described the results of observations made on the living spermatozoids of *Ginkgo*. He concluded that Hirase was in error in claiming the presence of a tail in *Ginkgo* and thinks the appendage supposed by Hirase to be a tail was a malformation due to compression in the escape of the spermatozoid from the pollen tube. Similar conclusions have been reached by Mr. Bessey (15), one of the writer's associates, after a careful examination of living material.

In July, 1899, Belajeff (14) brought forward further evidence to show that the blepharoplast must be considered a centrosome. He found by a careful study of *Marsilia* that the blepharoplast here not only occupies the pole of the spindle, but evidently takes part in spindle formation. He thus concludes that it is a veritable centrosome.

Strasburger, in his recent monograph (112) entitled "Ueber Reductionstheilung, Spindelbildung, Centrosomen, und Cilienbildner im Pflanzenreich," which appeared early in 1900, has again gone over the ground of swarm-spore and spermatozoid formation, and concludes that the blepharoplast of spermatogenous cells is distinct from a genuine centrosome or centrosphere, and traces its origin back to the "*Mundstelle*" of the swarm-spores of *Cladophora*, *Œdogonium*, etc., from which the cilia originate. His discussion of the matter is of the highest interest.

In 1900 the presence of spermatozoids in a third genus of the *Cycads* was proven. This was accomplished by Lang (77) in his investigation

of *Stangeria paradoxa*. The spermatozoids in this instance have not yet been studied in the living state and the details of the spermatogenesis have not yet been followed.

In the report of a paper read before the American Society for Plant Morphology and Physiology, published in Science, February 15, 1901, the writer (128) described the cell division giving rise to the stalk cell and central cell, and the morphology of the prothallial apparatus which has been entirely misinterpreted in all previous descriptions. The description there given is the same as that published in this monograph.

ACKNOWLEDGMENTS.

In concluding the introduction, the writer desires to express his indebtedness to various friends for aid furnished in the course of this investigation: To Dr. William Trelease, director of the Missouri Botanical Garden, who kindly aided him by furnishing developing seeds of *Ginkgo biloba* collected at regular intervals, and by general advice; to Mrs. L. H. Webber for considerable aid in the preparation of drawings; to Prof. E. C. Jaffrey and Dr. Erwin F. Smith for aid in the preparation of photomicrographs; and to Sir William Dyer, Prof. B. L. Robinson, and Mr. F. V. Coville for the privilege of examining and studying the species of *Zamia* in the Kew, Gray, and U. S. National herbaria, respectively. Lastly thanks are due to my colleague, Mr. Walter T. Swingle, who has aided me greatly throughout this investigation. Whatever merit the study may possess is, in a large measure, due to him.

METHODS AND MATERIALS USED.

The investigations have been limited mainly to the species of *Zamia* growing wild in Florida. *Ginkgo* was studied somewhat for comparison, but as this plant was being studied by Dr. Hirase, little time was given to its investigation. When the writer began the investigation of *Zamia* the forms growing in Florida were all generally referred to *Zamia integrifolia* Jacq. In the course of the investigations it was found that there were at least two distinct species in the State, neither of which could be considered as belonging to *Zamia integrifolia* Jacq., which is a West Indian species. One species is found very abundant on the east coast of Florida south of New River. This corresponded well with the description of *Zamia floridana* D. C., and a comparison of a fragment of specimen and a tracing in the Kew Herbarium of De Candolle's original specimen showed that the south Florida form must undoubtedly be referred to this species.[1] It has large elliptical,

[1] *Zamia floridana* D. C. (Prodromus 16, p. 544.) Leaves ovate or ovate-lanceolate, 20 to 30 cm. long, excluding the petiole; petiole about 20 cm. long, unarmed, triangular, sericeo-tomentose at base, with scattered hairs above; leaflets mostly opposite,

strongly umbonate cones 6 to 8 inches long and about 2½ inches in diameter.

The other Florida species is found along the east coast of the State from Titusville north to St. Augustine. Its southernmost extension on the east coast of the State, so far as known, is about 150 miles north of the northernmost extension of *Zamia floridana*. The writer's study of this plant seems to indicate that it must be referred to *Zamia pumila* L.[1] Some doubt remains, however, in regard to this, and it may ultimately prove to be an undescribed form.

In 1893 the writer first began the collection and preservation of Zamia material in preparation for a study of the spermatogenesis and embryology. The investigation can hardly be said to have commenced, however, until the appearance in 1896-97 of Hirase's and Ikeno's preliminary papers announcing the occurrence of spermatozoids in *Ginkgo* and *Cycas*. A study of the spermatogenesis and phenomena of fecundation was then commenced and carried on as rapidly as possible.

It was found by experience that cones of the two species could be wrapped in paper and shipped a two days' journey without noticeable

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smooth above, with scattered hairs below, 14 to 20 pairs, linear, 9 to 14 cm. long and 3 to 7 mm. wide, falcate and somewhat twisted, approximately erect, 10 to 16 nerved, narrowed at base, apex obtuse, with five or six obscure dentations, margin revolute; mature pistillate cones, oblong, 12 to 16½ cm. long and 6 to 8 cm. in diameter, markedly umbonate, densely tomentose, with persistent dark brown hairs; peduncle ferrugineous, tomentose, short, about 10 cm. long; seed-bearing scales peltate, hexagonal, thick and somewhat hemispherical at outer end; staminate cones, oblong, dark brown, tomentose, about 8 cm. long and 2½ cm. in diameter; peduncles short, 5 to 10 cm. long. Very abundant in southern Florida on the east coast below New River (latitude about 26° 30′). Inhabits open, comparatively dry pine forests (flat woods). Included in *Z. integrifolia* by Gray and Chapman. Not *Z. integrifolia* Jacq.

[1] *Zamia pumila* L. (in part). Leaves ovate, exclusive of petiole 20 to 30 cm. long; petiole unarmed, about 20 cm. long, triangular, sericeo-tomentose at base and with scattered hairs above; leaflets mostly opposite, but frequently irregularly placed, smooth above and with scattered hairs below, 16 to 22 pairs, linear-lanceolate, somewhat falcate, 7 to 11 cm. long and 8 to 16 mm. wide, mostly straight, but occasionally slightly twisted, 20 to 28 nerved, narrowed at base; apex obtuse, slightly serrate, margin revolute; mature pistillate cones, elliptical, scarcely umbonate, 6½ to 10½ cm. long by 5 to 8 cm. in diameter, densely tomentose, with ferrugineous, somewhat deciduous hairs; seed-bearing scales peltate, hexagonal, thin, and somewhat flattened at outer end; peduncle ferrugineous, tomentose, short, about 10 cm. long; staminate cones, oblong, brown, tomentose, about 8 cm. long and 2½ cm. in diameter; peduncle short, about 5 to 10 cm. long. Abundant in central Florida, particularly on the east coast between latitudes 28° 30′ and 29° 30′. Inhabits mostly dense moist woods (hammocks).

Z. pumila differs mainly from *Z. floridana* in its shorter and broader leaflets, which are less twisted and not so erect and rigid, and in its shorter nonumbonate cones with seed-bearing scales thinner and more flattened at outer end. It, furthermore, is very distinct in range and character of habitat

injury to the developing sexual organs. Arrangements were consequently made by which shipments of cones of the two species were received at regular intervals throughout the developing season. The cones of *Zamia floridana* were obtained at Miami, Fla. Two of these cones were mailed to the writer three times a week during the period found necessary to secure the desired stages. The cones of *Zamia pumila* were collected at New Smyrna and Daytona, Fla., and these were gathered and mailed to the writer twice a week, two or more being sent each time. In no case was the material injured in shipment so that any change in development could be observed. Cones remained fresh for a week or more after their receipt, and the normal process of pollen-tube development seemed to go on as usual until the seeds became very dry. This would normally be expected, as the developing pollen tubes derive their nutrition entirely from the nucellus, and the seeds are protected by their location, in the interior of the closed hairy cone, from any loss of moisture. All material fixed and utilized in the investigation was cut out and prepared immediately on the receipt of the cones, being taken thus about three days after the cones were cut from the plants. At several periods in the development visits were made to the regions where the plants grew, and absolutely fresh material was gathered and fixed in abundance for comparison. In all cases, however, material mailed to the writer gave results exactly the same as that cut and fixed in the field. In many cases cones were kept in the laboratory for one or two weeks after being received, and were examined at intervals to note how long they would remain satisfactory for study. In the examination of such cones the writer frequently found the spermatozoids living and moving in perfectly normal condition the same as those examined in the field a half hour after they were cut from the plant. The writer was located at Eustis, Fla., while the material was being shipped to him and prepared, but mail from Miami and New Smyrna required two days to make the journey, and could have been sent just as satisfactorily to Washington or New York. The facts regarding the shipment of the cones are given in some detail, as it is thought that *Zamia* should become a standard object of investigation and demonstration in the botanical laboratories of universities in the Eastern United States, and no trouble should be experienced in obtaining cones in good condition for study at any point within a three or even four days' railway journey of Miami, Fla., where abundant cones of *Zamia floridana* can be obtained. This is by far the best species for study, as a much larger percentage of the ovules are fecundated and a much larger number of pollen tubes are found developing in each ovule than in the case of *Z. pumila*. Further than this, the plants of *Zamia pumila* are more scattered, and it requires a considerable amount of work to secure any great number of cones. *Zamia floridana*, however, is very abun-

dant at Miami, and almost an unlimited number of cones can be secured any season. The following statement of dates at which time important changes take place in the developing organs of *Zamia floridana* may be of service in guiding investigators in the securing of important stages for investigation. It must be remembered, however, that different plants vary considerably in their stages of development, and the dates are thus only approximate.

(1) Pollination takes place the last of December and first of January.
(2) Germination of pollen and growth of prothallial apparatus from January 1 to June 1.
(3) The division of the second prothallial cell, giving rise to the stalk cell and central cell, occurs February 15 to March 10.
(4) The blepharoplasts first appear about March 1 to 20.
(5) The gradual development of the central cell blepharoplasts and prothallial apparatus continues from March 1 to May 30.
(6) The prophase of division of the central cell appears about May 20 to 25.
(7) Spermatozoids mature mainly between June 1 and 15.
(8) Fecundation takes place mainly between June 1 and 15.

In *Zamia pumila* the date of maturing of the spermatozoids and of fecundation in 1897 was fully three weeks later than in *Z. floridana*, and it is probable that this species is ordinarily considerably later. On the other hand, the date of maturity of the male cones, the pollination, and the first appearance of the blepharoplasts in the two species was found to be about the same.

When the cones were received the seeds were cut out and a portion of the apex of the nucellus 3 to 4 mm. in diameter, which contained the developing pollen tubes, was transferred as quickly as possible to the fixing solutions. In preparing the archegonia for study, cylinders about 5 mm. long and 2½ or 3 mm. in diameter were cut out of the apical portion of the prothallus containing the archegonia, and transferred to the fixing solution. Quite large portions of tissue must be used in this case from necessity, as the egg cells if cut into are destroyed for study, the protoplasm flowing out. In some cases portions of the seed were cut out and prepared, with the nucellus and prothallus in connection, to show the apparatus *in situ*, but this method is not satisfactory for the study of the finer cytological details of structure.

Various fixing agents were used in the course of the work, including Flemming's chromic-aceto-osmic acid solution, weak and strong, Hermann's platino-aceto-osmic acid solution, Van Rath's solutions II, III, and IV, chromic acid one-half per cent and 1 per cent, etc. Flemming's strong solution was used more than any of the other fixatives, and gave in general the best results. Its time of action was varied considerably, and in probably the majority of cases it was used diluted somewhat with water in the ratio of one part of the strong solution to two, four, nine, or nineteen parts of water. In general, a solution of one part to

four parts water gave very excellent results in fixing the pollen tube apparatus. In the fixation of the archegonia, however, at the time of fecundation and during the development of the embryos, it is necessary to use the fixative very strong, as it is difficult for the solution to penetrate the starchy matter of the prothallus which surrounds the archegonia.

In staining, the Flemming triple process with safranin, gentian violet, and orange G. gave by far the best results and was most extensively used. Heidenhain's iron-hæmatoxylin was also used considerably, and besides this Czoker's alum cochineal with Bismarck brown, fuchsin, and some other stains were occasionally used for comparison.

DEVELOPMENT OF THE MICROSPORES.

The pollen cones (figs. 3 and 4) of the two Florida species of *Zamia* begin to appear at the apex of the stem in July and continue to develop until the following January, when the pollen is discharged and pollination takes place.

The mature pollen grain examined in water is nearly spherical, but somewhat flattened on one side where the prothallial apparatus is attached. (Fig. 11.)

The earliest description of the pollen of *Zamia* known to the writer is that of Schacht (97) in 1860:

> Endlich hat Zamia ein kleines Pollenkorn mit einer sehr tiefen Längsfalte, welche sich in Wasser nicht ausgleicht. (Taf. XVII, F. 26 und 27.) Dieser Falte gegenüber liegt die kleine Tochterzelle, welche erst bei sehr gelungenen Querschnitten sichtbar wird (F. 28) und wahrscheinlich wie bei Cupressus nicht zur Ausbildung kommt, während die grössere sich als Pollenschlauch verlängert.

In 1872 Juranyi (72) described the structure and development of the pollen of *Ceratozamia longifolia*, which evidently corresponds closely with what occurs in *Zamia*. He found that two small cells were regularly cut off at one side of the large cell, and in some cases three. While the different cell stages of development are described and figured, the details of the division leading to the formation of the differferent cells was not followed.

In his study of the development of the pollen of certain Cycads, principally *Ceratozamia mexicana*, Guignard (45) was unable to confirm Juranyi's conclusion as to the occasional production of three prothallial cells. "According to the observations of M. Juranyi," wrote Guignard, "a third small cell may be formed by the division of this latter nucleus; but it does not appear to be so in the case of *Ceratozamia mexicana*."[1]

[1] "D'après les observations de M. Juranyi" wrote Guignard, "il peut se faire une troisième petite cellule, par suite de la division de ce dernier noyau; mais il ne paraît pas en être ainsi dans le *Ceratozamia mexicana*."

In the formation of the two prothallial cells, Guignard shows that in each case it is the nucleus of the large cell which divides in cutting off the small cells of the prothallus.

In the species of *Zamia* studied by the writer, the mature pollen grain always shows two prothallial cells cut off at one side, and protruding into it (fig. 11). The development of the pollen has not been carefully studied, and the details of the formation of the prothallial cells is not known. It seems from the writer's observation, however, that three cells are at least occasionally formed; and in this case the first one cut off is resorbed, as described by Strasburger (109) and others in *Pinus*, *Ginkgo*, etc., remaining as a dark more or less refractive layer in the wall of the pollen grain situated at the point of contact of the other cells (figs. 11, 13, and 14). In many instances of mature pollen grains, and in later stages, during germination, no indication of this resorbed prothallial cell can be observed, but in some cases it may be seen very plainly, and is unmistakable.

In *Ginkgo*, according to Strasburger (109) and Hirase (62), the nucleus of the pollen grain undergoes normally three divisions, by which three prothallial cells are cut off, the first of which becomes compressed against the wall of the pollen tube and is largely resorbed, in the mature pollen grain appearing very indistinctly as a slight layer in the wall (Strasburger, 109, Pl. I, figs. 5 to 7, and Hirase, 62, fig. 1).

A careful investigation of the development of the pollen of *Zamia* will have to be made before it can be determined whether three prothallial cells are regularly formed or whether the remnants of a third cell, occasionally observed, are to be considered as cases of rare and somewhat abnormal development. Judging from the normal occurrence of three cells in *Ginkgo* and *Pinus*, it would seem that probably three cells may also be normally formed in *Zamia*. However, in the mature pollen grain, and in the pollen grains after germination in the nucellus, a third cell can only occasionally be observed, and the description here given will deal mainly with the two prothallial cells plainly evident in all cases.

The nomenclature used here for the various cells of the antheridium is somewhat different from that usually used. It was thought best to use terms more in harmony with those used in the Pteridophytes in order to avoid confusion. The two prothallial cells normally cut off in the pollen grain are distinguished in the order of their formation as the first and second prothallial cells (P1 and P2). When the second prothallial cell divides it gives rise to the stalk cell and central cell (Körper cell, body cell, generative cell, etc.). The cell here called the central cell is considered by Strasburger (109, p. 7) and others as corresponding to the central cell of the antheridium in ferns. The

central cell when it divides gives rise to the spermatids, which become metamorphosed directly into spermatozoids. The entire apparatus, including the stalk cell and central cell (Körperzelle or generative cell) is spoken of throughout as the male prothallus, or simply prothallus where it is not necessary to distinguish more closely. The nomenclature here given corresponds with that used by Shaw in *Marsilia* (102).

In the case of *Ginkgo*, judging from Strasburger's and Hirase's figures, the walls of the prothallial cells cut off extend comparatively straight across the pollen grain, in each cell the new wall as formed being attached to that of the pollen grain. In *Zamia* quite a different form is found. Here the cells arch out into the tube cell of the pollen grain (fig. 11). In none of these cells is a cellulose wall laid down, there being nothing but a plasma membrane or hautschicht formed. The first prothallial cell is shaped like a plano-convex lens and arches out into the second prothallial cell. In the mature pollen grains it can not be determined whether the plasma membrane formed in cutting off this cell is attached at the sides to the plasma membrane of the tube cell, as must be the case if only two prothallial cells are formed, or to that of a third resorbed prothallial cell. In some cases of germinating pollen grains where the prothallial apparatus has developed considerably and where remnants of a resorbed third prothallial cell can be observed, the attachment would seem to be to the plasma membrane of this cell (figs. 13 and 14). This would also be indicated by Juranyi's figures of *Ceratozamia* (72, Taf. 33, figs. 8–11).

The second prothallial cell is attached to the first prothallial cell and arches out into the tube cell. Its membrane is connected only with that of the first prothallial cell. This cell, while the most important and ultimately much the largest, is in the mature pollen grain considerably smaller than the first prothallial cell. The protoplasm of the prothallial cells is densely granular and the nuclei, which nearly fill the cells, are difficult to distinguish. The nucleus of the tube cell is much larger than the nuclei of either of the prothallial cells and is situated at the apex of the prothallus.

The mature pollen grain of *Cycas revoluta*, as shown by Ikeno (70, Pl. VIII, fig. 13), would seem to be considerably different from *Zamia*, in that the cell membrane of the prothallial cell extends straight across the grain, as figured by Strasburger and Hirase in *Ginkgo*, instead of arching out into the tube cell as in *Zamia*. The structure described by the writer in *Zamia* is the same as that described by Juranyi (72) and Guignard (45) as occurring in *Macrozamia*.

Pollination in both of the species of *Zamia* studied apparently takes place in the latter part of December and first of January, and is accomplished mainly through the agency of the wind. The pollen is produced in great abundance and is light and easily carried. The scales

of the female cones throughout their existence, except at the time of pollination, are tightly closed, so that no dust can gain admission to the interior (figs. 2 and 6). When the cone is receptive and ready for pollination the basal scales of the cone separate from those above, leaving a crack about one-eighth to one-fourth of an inch wide between them. This crack extends around the entire base of the cone and apparently remains open at least a day or more, though the time has not been determined by actual observation. When the ovules are pollinated, apparently the row of scales immediately above this move downward, closing the original crack and leaving a similar opening between them and the row of scales next above. This process evidently continues in succession, following the spiral arrangement of the scales, until the top is reached and all of the ovules have been pollinated. Several days are evidently consumed in the process of pollination of a single cone. The scales evidently reverse in quite regular order, as the opening between them is never found here and there over the cone, but always in a continuous and quite regular crack running around the cone. A single scale does not remain open longer than others because its ovules have not been pollinated, as might be supposed from the fact that almost universally among plants the style of a pistil which has not been pollinated remains fresh and the stigma receptive, and persists for a much longer period than in one which has been pollinated. The effect of pollination in *Zamia* seems to have no influence on the length of time during which the scales remain open or on their endurance. The writer has found many cones developing normally for several months after pollination in which only a few seeds had set, and frequently mature cones have been found containing only two or three seeds. This infertility is doubtless due to the lack of pollination, as it has only been found in the case of *Z. pumila* at New Smyrna and Daytona, where microscopic examination has revealed a decided lack of pollen, many ovules being frequently found in a cone without a trace of pollen or pollen tubes. The plants of this species in these regions are scattering and pollination is frequently very imperfect.

Z. floridana in the regions studied is very fertile, almost every ovule being fecundated and maturing a perfect seed.

DEVELOPMENT OF PISTILLATE CONES.

In the present paper the structure and development of the pistillate cones (figs. 2 and 6) will be discussed only so far as it bears on the question of development of the pollen tubes and fecundation. At a later time the writer hopes to describe the development of the archegonia more in detail.

At the time of pollination the ovules are about 1 cm. long by 5 mm. broad (fig. 2). The single coat of the naked ovule is considerably

thickened at the apex, and the micropyle through which the entire pollen grain must pass forms a continuous tube from the surface to the apex of the nucleus, a distance of about 3 mm. The micropyle at the apex of the ovule may be seen with the unaided eye as a small round hole somewhat smaller than the diameter of an ordinary pin—about one-fourth millimeter (figs. 1 and 9).

The nucellus at this time is about 2 mm. in diameter and pointed at the apex. Shortly before pollination the tissue at the apex of the nucellus was found to be solid entirely to the point; but just before or during pollination a cavity, the pollen chamber for the reception of the pollen, is formed in the apex by the breaking down of the tissue (fig. 5). The pollen grain to be effective must pass through the entire length of the micropyle and finally come to lie in this chamber. It is difficult to understand how the nonmotile pollen grains can ever reach the pollen chamber, which would seem to be absolutely safe from infection by them. It is easy, however, to see how a few grains may be wafted by the wind into the cone, when the scales separate as above described, and rattle down to the axis of the cone, around which the apexes of the ovules are crowded.

The passage of the pollen grain through the micropyle is evidently accomplished by suction. A mucilaginous, stigmatic, or micropylar fluid is secreted by cells of the ovule coat surrounding the micropyle, and this is evidently protruded in a drop from the micropyle at the time of pollination as a trap for the pollen. This secretion has at least been observed several times by the writer protruding from the opening of the micropyle at about the time of pollination, and its formation is thought to be of normal occurrence. This secretion later disappears, and a suction is probably formed by the breaking down of the cells in the formation of the pollen chamber which leads to the fluid, together with any pollen grains which have come in contact with it, being drawn down into the required position in the pollen chamber. The gradual absorption of the fluid by the cells of the nucellus bordering the pollen chamber would, of course, accomplish the same result. The breaking down of the tissue at the apex of the nucellus in the formation of the pollen chamber occurring about this time would seem to have some significance of this sort, and is believed by the writer to unquestionably be connected in some such way as above described in securing the passage of the pollen grains to the nucellus. In reaching the entrance to the micropyle of the ovule the pollen grains largely follow the trend of gravity. The passage of the micropyle, however, which is but slightly larger in diameter than the pollen grains, must be made against the action of gravity, and some such explanation as the above is necessary to understand how it can be accomplished. In *Ginkgo* and *Cycas* the pollen must pass through a similar long and narrow micropyle, and some such method of pollination must occur.

At the time of pollination in January the prothallus forms a spherical mass of soft, watery, rapidly-developing tissues in the middle of the nucellus which still comprises a considerable thickness of tissue on each side (fig. 1). No trace of the archegonia can yet be discovered.

The ovule at this time has reached only about one-third of its mature width and length, and growth in the size of all organs continues for a considerable period following pollination. The prothallus grows in size proportionately more rapidly than the other organs, and this is accomplished largely at the expense of the nucellus, which gradually becomes thinner throughout, and is finally, at the time fecundation occurs, found to be compressed to a very thin membrane at the apex, and below on the sides and base has largely split up into very thin shreds, seldom being found as an unbroken membrane throughout.

The archegonia are differentiated in the upper part of the prothallus shortly after pollination, but do not reach their mature size until a short time before fecundation, which does not occur until four months later. Four archegonia are almost universally formed in each prothallus, but some instances have been observed where a fewer (2 or 3) or a larger number (5 or 6) have been developed.

During the increase in size of the archegonium through the months of March, April, and a part of May, the nucleus of the central cell remains in the upper part of the cell near its point of reorganization after the preceding division, which gave rise to the neck cell (fig. 10). It is usually elliptical and very large in comparison with the nuclei of surrounding cells. This location of the nucleus during the main growth period of the central cell of the archegonium is evidently common in related plants until after the ventral canal cell is cut off. Treub described the same location in *Cycas circinalis* (117), Ikeno in *Cycas revoluta* (65), Hirase in *Ginkgo* (59), Blackman in *Pinus sylvestris* (16), and Murrill in *Tsuga canadensis* (91, p. 587).

The protoplasm of the central cell during the latter part of this period of growth in size presents the most beautiful foam structure the writer has ever observed.

Shortly before fecundation the nucleus of the central cell divides and a small cell is cut off at the apex, which corresponds to the ventral canal cell of the conifers. Until the publication of Ikeno's preliminary note announcing the discovery of this canal cell in *Cycas revoluta* (65) it had been supposed that it was not formed in the Cycadaceæ. It would seem, however, from its occurrence in *Cycas* and *Zamia* that it is probably as generally formed in the Cycadaceæ as in the Coniferæ. Hirase has also recently described the formation of this cell in *Ginkgo biloba* (59).

s not observed the division of the nucleus leading to
e canal cell in *Zamia*, but the process probably cor-

responds very closely to that occurring in *Cycas*, *Ginkgo*, and the Coniferæ. The nucleus of the central cell in preparing for division evidently goes through changes similar to those described by Murrill in *Tsuga canadensis*, and it would be interesting to know if the same unique method of spindle formation occurs in *Zamia* also. The accumulation of highly granular cytoplasm in a conspicuous mass below the nucleus of the central cell, as described by Murrill in *Tsuga*, is uniformly found in stages immediately preceding division in *Zamia*. The synapsis condition observed by Murrill in an early prophase of the division in *Tsuga* is also of common if not normal occurrence in *Zamia*, so that it would seem probable that the spindle formation in *Zamia* may be similar to that of *Tsuga*. It is interesting to observe that the spindle formed in this division is strikingly blunt-poled, as observed by Ikeno in *Cycas* (65) and Blackman in *Pinus* (16).

Before fecundation the canal cell breaks up and loses its identity, only traces of it being occasionally found at the time of fecundation. After the division giving rise to the canal cell is completed, the lower nucleus which forms the oosphere travels from the apex of the cell downward toward the center and takes a position slightly below the middle of the cell, where it remains until fecundation takes place. It is usually spherical or slightly elliptical, and its contents are much less dense than the surrounding cytoplasm of the egg cell, with which it forms a marked contrast. The mature egg cell is usually elliptical or slightly reniform and is about 3 mm. in length and from 1 to 1.5 mm. in width. The nucleus is very large, being about 553 μ long and 467 μ in diameter. It is plainly visible to the unaided eye in stained sections and it is hard to realize on looking through a section held up to the light that one is viewing the egg cell and its nucleus without even the use of a hand lens.

In the development of the prothallus a circular depression known as the archegonial chamber (prothallial or endosperm cavity) is formed in the upper part of the prothallus immediately above the archegonia and beneath the apex of the nucellus (fig. 9).

This cavity is usually about 2 millimeters in diameter and a millimeter deep. It is into this cavity that the pollen tubes later grow and discharge their spermatozoids. The openings to the four archegonia can be seen easily in the bottom of the cavity, the two neck cells being turgid, hyaline, and quite distinct in appearance from the surrounding cells of the prothallus. They protrude above the general surface and appear to be under considerable tension.

DEVELOPMENT OF THE POLLEN TUBE AND PROTHALLUS.

GERMINATION OF POLLEN AND GROWTH OF PROTHALLUS.

Very shortly after the pollen grains have been drawn down into the pollen chamber of the nucellus they germinate, the tube, which at first is about the diameter of the pollen grain or slightly less, bursting out

of the exine of the grain at a point opposite the attachment of the prothallus. No matter in what direction the pollen grain may lie, the tube as soon as protruded grows toward the tissue of the nucellus, forming the side of the pollen chamber, into which it soon penetrates (fig. 5).

If the pollen grain is so situated that the tube when first protruded points toward the apex or base of the nucellus, it makes a sharp turn immediately after leaving the pollen grain and enters the nucellar tissue. In *Zamia* the tube never branches before entering the tissue, and while it occasionally branches after entering the tissue, this is by no means of common occurrence. The majority of tubes remain unbranched throughout their growth. In *Gingko*, on the contrary, the distal end of the tube as soon as it enters the nucellar tissue becomes very much branched and the ramifications are so slender that it is only with the greatest difficulty that they can be traced.

When the pollen tube first ruptures the exine and protrudes, it is considerably smaller than the diameter of the pollen grain. The relation of the prothallial cells and tube cell which forms the tube is shown in this stage in figure 12. As the tube pushes out, the protoplasm of the tube cell draws away from the wall of the old pollen grain to some extent apparently in all instances, though it would seem probable that the contraction shown in the figure is somewhat abnormal. The nucleus of the tube cell, which is densely granular, immediately passes into the tube, becoming the pollen tube nucleus, and travels farther as the tube grows, remaining always about a uniform distance from the apex of the tube. In this early stage of germination the cells of the prothallus have the same size as in the mature pollen grain and appear about the same.

The entire prothallus in this stage is about 9 μ wide and 8 μ long (length being considered the extension in the direction of the growth of the pollen tube). The nuclei in both the first and second prothallial cells are still very densely granular and almost fill up the entire cell in each case. The nuclei in both cells are more or less crescent-shaped in median section, corresponding to the shape of the cells. In the tube shown in figure 10 the nucleus of the first prothallial cell measured 7.12 by 3 μ; that of the second prothallial cell 8 by 3 μ, and the nucleus of the tube cell 8.01 by 5.34 μ, the nucleus of the tube cell in this stage always being slightly larger than the nuclei of either of the prothallial cells.

When the Flemming triple stain was used in the study of pollen tubes the safranin always stained the wall of the pollen grain red, serving as an important distinction for a pollen grain wall throughout the development of the apparatus.

Before the pollen tube has increased in length very greatly it increases also noticeably in width, and by the time it has reached a length four or five times as great as the diameter of the pollen grain

it has reached a diameter as great as that of the pollen grain (fig. 13). The point where the tube bursts out of the pollen grain has also enlarged, the broken edges of the exine being bent outward. Meanwhile the tube nucleus has assumed a round form, increasing somewhat in size.

The protoplasm presents a beautiful foam structure, with large vacuoles here and there. The starch grains which later fill the tube have not yet begun to appear.

The next noticeable differentiation in the growth of the tube is the increase in size of the cells of the prothallus. Both cells increase in width and length and the first prothallial cell pushes out into the second prothallial cell, which becomes shaped like a concavo-convex lens and is crescent-shaped in cross section. Figure 14 shows a pollen tube in the first stage of the development of the prothallus. The prothallus here has reached a size of 15 μ wide by 16 μ long. The nuclei of both prothallial cells have increased slightly in size and become spherical and less densely granular. The pollen tube is meanwhile gradually growing in length and diameter, the tube nucleus passing farther down as the tube elongates. In this tube (fig. 14) a dark line appears at the base of the prothallus which seems undoubtedly to be the remains of a third prothallial cell which has been resorbed.

The pushing out of the first prothallial cell into the second prothallial cell is a point of considerable interest in clearing up the morphology of the prothallial apparatus, which was left in a very unsatisfactory state in the writer's preliminary papers, as well as in the papers of Ikeno (70) and Hirase (62). In a somewhat later stage, when both of the prothallial cells have reached almost twice the size described in the last-mentioned stage, the first prothallial cell can be seen to have pushed a considerable distance into the second prothallial cell, the point of attachment of the plasma membrane of the cells still remaining in about the same relative position as in the original pollen grain (fig. 15). Meanwhile the second prothallial cell has arched out still farther, and by the increase in size of the first prothallial cell has been carried mainly out of the old walls of the pollen grain into the pollen tube. It may be remarked here that the prothallus still retains its original connection with the wall of the pollen grain, a connection which remains unbroken until the spermatozoids mature. In the preceding stage the nucleus of the second prothallial cell had increased in size slightly more rapidly than that of the first prothallial cell and had become slightly larger (fig. 14). In this stage (fig. 15) the second prothallial cell nucleus has become decidedly larger than that of the first prothallial cell.

DIVISION OF SECOND PROTHALLIAL CELL.

Shortly after this stage the second prothallial cell divides into two very unequal cells, the stalk cell and central cell (körper cell, generative

cell, etc.). In his early studies the writer concluded from analogy with the development of the gymnosperms as described by Belajeff (2 and 3), Strasburger (109), and others, that a division of the second prothallial cell must take place. It, however, was only after prolonged and diligent search that the evidence establishing this fact was finally secured, and only a single tube has been found in all the many examined where the presence of a division was evident. This, however, was fortunately in just the stage to settle the disputed point. It is in a telophase of the division when the two-daughter nuclei are reorganizing and the spindle connecting them is yet clearly evident (fig. 17). The first prothallial cell extending into the second prothallial cell is here clearly distinguishable, as in the preceding case described (fig. 15). The spindle, from the crescent shape of the second prothallial cell, assumes a position at an angle to the major axis of the prothallus, the lower nucleus and end of the spindle being crowded to one side by the position of the first prothallial cell, while the upper nucleus occupies a central position in the upper half of the cell which, when the new wall is formed, will become the central cell. The lower nucleus, which becomes the nucleus of the stalk cell, is already in this early stage noticeably smaller than the upper nucleus. Several round bodies which take a brilliant safranin stain in the Flemming triple process, and are evidently masses of nucleolar matter, are situated in the cytoplasm just outside of the spindle. The reorganizing daughter nuclei, in the only section secured in this stage, are too densely stained to show their structure well; they appear simply to be densely granular. The spindle fibers show very plainly, but do not as yet show any thickenings in the center preparatory to the formation of a cell membrane. A most careful search has failed to reveal any suggestions of a centrosphere or centrosome at the apex of the spindle where one might be expected to occur. In a number of instances a careful search has been made in the second prothallial cell, when it approaches division, for evidence of the presence of organs resembling blepharoplasts or centrosomes. Occasionally small centers with a few radiations have been observed (fig. 16), but these are irregular in their appearance and would seem to have no relation to blepharoplasts or centrosomes. The pollen tube at this time has reached a length of over 1 millimeter and starch grains have begun to appear, two being shown in the tube figured. Cells in the path of the tube are broken down and absorbed, apparently very little or no trace of them remaining.

In *Z. floridana* in 1898 the division of the second prothallial cell was found to take place mainly between February 15 and March 5. In *Z. pumila* the same year the corresponding division occurred between February 25 and March 15.

htly later than the above the central and stalk
parated by a plainly visible plasma membrane
st above the apex of the first prothallial cell

(fig. 18). This stage is a very easy one to find and the writer has many sections showing it very plainly. In the tube shown in figure 18, which is only shortly after the completion of the division, the nuclei of both the stalk and central cells have assumed a rounded form, the latter being much the larger. The nucleus of the central cell is here 9.79 μ in diameter while that of the stalk cell is only 7.12 μ and that of the first prothallial cell about 8.9 μ in diameter. The entire prothallus in this stage immediately after the division is only 29.37 μ long by 16.91 μ wide. The first prothallial cell is now almost entirely surrounded by the stalk cell, only the base of the cell remaining in its original position. A few small starch grains have already begun to appear in this cell, two being shown in the figure. In later stages both this cell and the stalk cell become crowded with starch. Before proceeding farther it will be desirable to point out the views held in regard to the structure of the prothallus in Cycadaceæ and *Ginkgo* in previous publications. Attention was first called to the peculiar structure of the prothallus in the Cycadaceæ in the writer's first preliminary paper on *Zamia* (122). Here it was stated:

> The former cell [in reality the stalk cell, as proved by later researches] is spherical or slightly elongated and presents a most singular structure. The nucleus of the original cell evidently divides into two, and one of the daughter nuclei forms within the unbroken *Hautschicht* of the mother cell a new and wholly distinct *Hautschicht*, which delimits a cell lying entirely free within the mother cell and surrounded on all sides by a layer of protoplasm of nearly uniform thickness (figs. 1a and 2). The other daughter nucleus remains free within the *Hautschicht* of the mother cell, but is pressed to one side by the interior cell.

It will be seen from this that the writer was greatly in error in his early interpretation. This was largely due to the fact that sections must be exactly median longitudinal through the pollen grain and prothallial apparatus to show that the first prothallial cell (interior cell) has any connection with the wall of the pollen tube. Cross sections of the tube which were then used considerably in the writer's investigations also fail to show the true relationship of these cells. Their confusing structure in a section of this kind will be seen by examining figure 23. The difficulty of the investigation leading to the correct interpretation of this structure is also shown by the views expressed by the Japanese authors which are at great variance with those of the writer.

In *Ginkgo* the first prothallial cell, which the writer has found to become surrounded by the stalk cell through transformation during growth, Hirase considers to be simply strands of protoplasm in the second prothallial cell. He says:

> At the extremity of the tube, which is covered by the exine and extends into the cavity, are found in the interior two flattened prothallial cells which are now separated from each other. Between these there are large vacuoles, and it may be seen also that they are united by the cytoplasm which forms two hollow cylinders placed one within the other, so that if a section be made along the longitudinal

axis of a pollen tube appearing at this stage, the two cells appear to be joined together by four cytoplasmic filaments. This condition of the tube continues up to the moment of fecundation.[1]

In the further development of the prothallium Hirase states that the second prothallial cell divides, and without forming a partition wall one of the naked nuclei is crowded out of the cell into the first prothallial cell, coming to be located outside of the interior protoplasmic strands. He says:

In the middle of July the nucleus of the interior cell [second prothallial cell] above mentioned divides into two daughter nuclei. I have not had an opportunity to observe the karyokinesis of this division * * *. Immediately after their division, one of the nuclei becomes much larger than the other and proceeds to occupy the central part of the mother cell, increasing in size more and more. On the other hand, the smaller of the two daughter nuclei leaves the mother cell, or, rather, is expelled from it by the other, and proceeds as far as the space between the two cytoplasmic cylinders which connect the mother cell with the posterior prothallial cell.[2]

The naked nucleus which after the division of the second prothallial cell is crowded out of this cell into the first prothallial cell he considers to be the equivalent of the stalk cell or *stielzelle* of the Coniferæ, and the cell from which it is expelled he says corresponds to the *Körperzelle* (central cell).

Ikeno's description of the development of this stage in Cycas (70, p. 570) corresponds in all important points with that given by Hirase for Ginkgo. He wrote:

While the latter cell [second prothallial cell] has become somewhat extended and is still globular, its nucleus divides into two daughter nuclei of equal size. * * * A septum between these daughter nuclei is never formed. One of them only expands rapidly and occupies the larger space of the mother cell, so that the other cell is immediately expelled from it in a naked state.[3]

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[1] À l'extrémité du tube qui est couverte par l'exine et fait saillie dans la cavité, on trouve à l'intérieur deux cellules prothalliennes aplaties qui sont maintenant séparées l'une de l'autre. Entre elles, sont de grandes vacuoles et on voit aussi qu'elles sont unies par le cytoplasme qui forme deux cylindres creux placés l'un dans l'autre, de sorte que si l'on coupe selon son axe longitudinal un tube pollinique parvenu à ce stade les deux cellules semblent être réunies ensemble par quatre filaments cytoplasmiques. Cet état du tube persiste jusqu' au moment de la fécondation. (62, p. 109.)

[2] Au milieu de juillet, le nucléus de la cellule intérieure [second prothallial cell] susdite se partage en deux nucléus-fils. Je n'ai pas eu la chance de pouvoir observer la karyokinèse de cette division * * *. Aussitôt après leur formation, l'un des nucléus devient beaucoup plus gros que l'autre et vient occuper la partie centrale de la cellule-mère en grossissant de plus en plus. Au contraire, le plus petit des deux nucléus-fils quitte la cellule-mère ou mieux en est refoulé par le plus grand et s'achemine jusqu'à l'espace compris entre les deux cylindres cytoplasmiques qui joignent la cellule-mère et la cellule prothallienne postérieurs (62, p. 110).

[3] Während die letztere Zelle [second prothallial cell] etwas ausgewachs
noch kugelig bleibt, theilt sich ihr Zellkern zu je zwei Tochterkernen
Grösse. * * * Eine Scheidewand zwischen diesen Tochterkerne
gebildet. Einer von ihnen nur wächst schnell aus und nimmt den
theil der Mutterzelle ein, so dass der andere alsbald im nackten
verdrängt wird.

Ikeno also considers the naked nucleus expelled to be the homologue of the Stielzelle or stalk cell and the cell from which it was expelled the homologue of the Körperzelle. It does not appear from Ikeno's monograph that he observed the division of the third prothallial cell leading to the formation of this structure. Judging from his short description and indefinite figures it would seem that his material at this stage must have been poor or lacking. In none of his figures of the male prothallus does he show an interior cell like the writer's first prothallial cell or strands of protoplasm such as Hirase describes in *Ginkgo*.

The two nuclei in his "first prothallial cell" are in position exactly the same as the corresponding nuclei in *Zamia* and *Ginkgo*, but no strands of protoplasm or cell membrane separates them. The "Körperzelle" in his figures 15 to 19a is indicated as entirely spherical and not influenced in shape at the attachment with the prothallial cell, which seems very unlikely. The writer is unable to suggest how this apparent difference between *Cycas* and *Zamia* can be explained. The series of *Zamia* preparations on which his interpretation is based has been shown to several American botanists, and they entirely concur with him as to the structure of *Zamia*.

The development of the prothallial apparatus of both *Zamia* and *Ginkgo* has been studied by the writer with considerable care at different times during a period of nearly four years, and with abundant material at different stages. The interpretation given by Hirase and Ikeno seemed so novel and improbable that he was stimulated to a more thorough investigation. The early studies of Juranyi (72), etc., give no aid in this question, as in his study of *Macrozamia* he germinated and grew the pollen on soft pieces of pear fruit, and it has been amply demonstrated since, that the developments obtained in this way were abnormal. Juranyi obtained fairly long tubes developed from the large pollen cell, traced the nucleus in its passage into this tube, and in two instances found that this nucleus had divided into two. The so-called Innenkörper (the prothallus), however, remained in its place, decreased in size as the tube elongated, and finally disappeared. Strasburger (110) cultivated pollen of *Ceratozamia* in the same way, and found that the Innenkörper did not disappear as long as the tubes remained in apparently a normal healthy condition. Belajeff (2 and 3) was the first investigator to introduce the only safe method, that of studying the pollen tubes developed on the pistil in the normal way, by sections of the pistil and isolating the tubes by maceration methods. His study of gymnosperms, however, did not extend to any of the *Cycadaceæ*.

In 1892 Strasburger described the development of the pollen and pollen tube of *Ginkgo*, but was led to erroneous conclusions, apparently, by the insufficiency of his material. He correctly described

and figured the pollen tube and prothallus in an early stage still showing the two prothallial cells in position. In describing the further development, however, he says:

> It is thus shown that in the second half of September the first of the two prothallial cells divides into a body cell and a stalk cell, while the outer prothallial cell usually remains undivided. The body cell corresponds to the central cell of an antheridium; it increases in size more than double, and its nucleus is enlarged in the same proportion. Hereupon this central cell undergoes commonly a cross or oblique division, by means of which two generative cells are created. The stalk cell of the antheridium is divided apparently only under certain conditions. Then the stalk cell and the first prothallial cell lose their independence, and the liberated generative cell passes into the pollen tube.[1]

This method of development would make *Ginkgo* correspond nicely with what occurs in some of the Coniferæ, but would seem to be quite different from what actually occurs in *Ginkgo*.

The writer's investigation of *Ginkgo*, so far as carried out, indicates that the development of the prothallus here corresponds entirely with that described above in *Zamia*. Shortly after germination the first and second prothallial cells can be discovered in the process of extension, the first protruding considerably into the second. In *Ginkgo* the writer has not been fortunate enough to find the division of the second prothallial cell, which gives rise to the stalk cell and central cell. The three-celled stage immediately following the division, however, compares almost exactly with the three-celled stage of *Zamia* (compare figs. 15 and 18), showing the first prothallial cell protruding into the stalk cell, and almost entirely surrounded by it. In older stages, both in *Ginkgo* and *Zamia*, when the central cell approaches the time for division, the first prothallial cell is almost invariably found to have grown up within the stalk cell to such an extent that it comes in contact with the central cell (fig. 20). In *Zamia* several instances have been observed where it has even caused a decided indentation in the central cell (fig. 22). Another feature of importance in showing that what the writer calls the first prothallial cell is a genuine cell and not simply a central portion of a cell inclosed by protoplasmic strands is shown in the fact that, in some cells as a result of fixation or a different stage of development, the plasma membranes or *Hautschichts* of the two adjoining cells separate, so that one can clearly distinguish two dis-

[1] So zeigt es sich denn, dass in der zweiten Hälfte des Septembers die vordere der beiden Prothalliumzellen in eine Körperzelle und eine Stielzelle zerfällt, während die äussere Prothalliumzelle gewöhnlich ungetheilt bleibt. Die Körperzelle entspricht der Centralzelle eines Antheridiums, sie schwillt zum mehr als Doppelten noch an, und in demselben Maasse vergrössert sich ihr Zellkern. Hierauf erfährt diese Centralzelle schon vielfach eine quere oder schräge Theilung, wodurch zwei generative Zellen geschaffen werden. Die Stielzelle des antheridiums scheint sich nur unter Umständen zu theilen. Dann geben Stielzelle und erste Prothalliumzelle ihre Selbständigkeit auf, und die befreite generative Zelle wandert in den Pollenschlauch ein. (109, p. 18).

tinct membranes (fig. 19). The plasma membrane delimiting the cells in this case stain the same and appear the same in all noticeable characters as the membranes in other portions of the same cell and of the central cell.

APPEARANCE AND GROWTH OF BLEPHAROPLASTS.

After the division of the second prothallial cell into the stalk cell and central cell the entire apparatus continues to grow in size, and the next important stage of development following this is the appearance of the blepharoplasts.[1]

In order to determine the true nature of the blepharoplast it was necessary to know its history, and a very careful study has been made of its first appearance and gradual development. They were first discovered by the writer in *Zamia* in a medium stage of development, as shown in figures 26 and 58, in which stage they present a very striking appearance and would be taken for undoubted centrosomes. Similar organs occur in the central cell of *Ginkgo biloba*, and were first described by Hirase in 1894 (57). Hirase simply described their appearance in a half-grown stage, without tracing out their origin and function. They were next described by the writer in *Zamia* in 1897 (122, 123, and 124), and here their gradual growth and development into the cilia-bearing organ of the spermatozoid was traced. It was further found that they had no intimate connection with fecundation, being left at the apex of the egg cell while the nucleus passes on alone and fuses with the egg nucleus.

Not being able to obtain material of *Zamia* in 1897 to trace out the origin of the blepharoplasts, the early stages of *Ginkgo* were studied, and it was found that here they were formed *de novo* in the cytoplasm of the central cell. In 1898 the same organs in *Cycas revoluta* were carefully described by Ikeno (70). Since publishing his results in 1897 the writer has made a very careful study of the early stages in *Zamia*, and finds that here also they originate *de novo* in the cytoplasm of the cell, as first described by him in *Ginkgo*.

During the division of the second prothallial cell as pointed out above (fig. 17), no indication of any organ resembling a blepharoplast or centrosome could be discovered at the pole of the spindle. The difficulty of obtaining this cell in stages of division, however, has prevented a very thorough examination at this stage. After the division is completed in stages like that represented in figure 18 and slightly later, a very careful examination fails to reveal a trace of any organ which could be considered to be an early stage of the blepharoplast. When

[1] A term applied by the writer (124, 1897), to the cilia-forming organ of the spermatogenous cells of *Zamia* and *Ginkgo* which so nearly resembles a centrosome or centrosphere. The term is derived from βλεφαρις, eyelash or cilium, and πλαστος, formed.

the central cell has increased in size to about twice the diameter it had immediately after its reorganization, the blepharoplasts first begin to appear. (Compare figs. 19 and 24.) In *Zamia floridana* in 1898 the blepharoplasts appeared mainly between March 5 and March 15, while in *Z. pumila* the same year they appeared mainly between March 10 and 25. It is probable that the date of their appearance may vary somewhat in different years, and the time of their appearance in different plants and even in different ovules of the same cone is very variable. Indeed, great difference has been noted in the time of their appearance in different pollen tubes in the same nucellus. It is interesting to note that the pollen tubes have considerable individuality apparently, and vary greatly in their stage of development, size of organs, etc. In tubes on the same nucellus the writer has found in some the fully developed spermatozoids, while in others the central cell had not yet divided.

In the earliest stage in which the writer has been able to surely recognize the blepharoplast, it seems to be made up of a small, deeply-staining granule from which several filaments of kinoplasm radiate, following the meshes of the reticulum. The central granule does not seem to be different in substance from the radiations—stains the same and shows no differentiation of structure. In this stage it is only a half micron in diameter or less and seems to be scarcely more than the point of crossing of the filaments of kinoplasm. They are located in the cytoplasm about halfway between the nucleus and the cell wall. Two are formed in each central cell at the same time and apparently independently. They are commonly located on opposite sides of the nucleus, but in a number of cases in this stage and in a still later stage they have been found nearer together, frequently less than 45° apart (figs. 19 and 25). The cytoplasm at the time the blepharoplasts appear forms a loose, open, reticular structure, and the rays which extend out from the blepharoplasts seem to run into the walls of the reticulum. The rays in this early stage are comparatively few and short. The nuclear plasm shows a reticular structure much finer than that of the cytoplasm and surrounds a large nucleolus. In the several instances of this early stage of the blepharoplast which have been observed they are located about midway between the nuclear membrane and the cell wall. In what seems to be a slightly later stage, however, when the blepharoplasts have grown considerably in size and show a distinct spherical body at the point of the converging rays, they are found quite close to the nuclear membrane, which is commonly slightly indented just below them (fig. 19).

In *Cycas*, according to Ikeno (70, p. 571), the two blepharoplasts appear in the central cell shortly after the division which gives rise to this cell and the [illegible] arise as two small bodies which at first lie close to [illegible]eus. No radiations are visible

from them for a considerable time. In this latter feature they seem to be considerably different from *Zamia*, where the radiations are visible and conspicuous in the youngest stage which can be detected. In *Ginkgo*, also, as shown by the writer (124) and Hirase (62), the blepharoplasts appear in the central cell just after its formation, arising in the cytoplasm near the nuclear membrane. It is probable that *Zamia*, *Cycas*, and *Ginkgo*, agree in their main features, the absence of radiations in *Cycas* being probably due to the method of preparation.

In this stage of development in *Zamia* the central cell is still almost spherical, being flattened at the point of attachment with the stalk cell, as is shown in figure 19. Here the central cell is only about 36 μ in diameter, while the nucleus is about 18½ μ, the nucleolus 4½ μ, and the blepharoplasts 1 μ in diameter. The first prothallial cell is shown here very plainly, pushing up into the stalk cell, extending fully two-thirds of the distance through it. The nucleus of the prothallial cell and that of the stalk cell are about the same size, the latter nucleus having increased in size since the stage illustrated in figure 18, and become somewhat compressed and lenticular from pressure. The pollen tube at this stage has reached a length of about 1 mm., and is at most places in the tissue from 40 to 50 μ in diameter, though this varies considerably. The protoplasm forms an open foam structure with large vacuoles, about the same as illustrated in figure 19. Starch grains have already become abundant in the tube, but are not so large or so numerous as in later stages. In the section figured no starch grains were visible in the stalk cell or prothallial cell, although in some instances they are formed in a still earlier stage.

The first indication of differentiation in the blepharoplast, as it increases in size, is the formation of an outer membrane or wall (fig. 25). By this time the blepharoplasts have moved somewhat farther away from the nucleus and the kinoplasmic radiations have become much longer, more prominent, and apparently more numerous. The central cell has also increased in size, as well as the entire prothallus. Up to this time the central cell has been nearly spherical in outline, and the blepharoplasts, so near as the writer has been able to observe, did not seem to occupy any definite position in reference to the attachment of the stalk cell. As the apparatus increases in size the central cell elongates and becomes elliptical or oblong, its major axis corresponding to the longitudinal axis of the pollen tube (fig. 20). The blepharoplasts during this development take up a position on opposite sides of the nucleus almost exactly on the major axis of the cell. In almost all of the cells in this stage of development the blepharoplasts occupy this position. In a number of instances, however, they have been observed to remain much closer together, and in some instances never assume a position opposite each other. In this early stage of development the central cell presents a beautiful reticular

structure (fig. 26), the meshes of the reticulum being rather large. The kinoplasmic filaments are but little more prominent than the walls of the reticulum into which they seem to run and disappear. The individual filaments themselves seem to be composed of fine granules and are, the writer thinks, quite surely threads and not plates. By carefully focusing above or below the blepharoplasts, irregularly arranged granules are seen which seem to indicate the thread-like nature of the radiations. These granules are interpreted as being cross sections of the kinoplasmic filaments. This, the writer is aware, is a disputed point in the structure of the kinoplasmic rays, and he has therefore very carefully examined many slides in the hope of being able to settle this question, at least in *Zamia*. The radiations are larger and coarser in *Zamia* than in any other plant which has come under the writer's observation, and it would seem to be a very favorable subject for the study of such disputed points. The cytoplasm, viewed in cross or longitudinal section, presents the same irregular meshes, and while the writer is inclined to view this as a foam structure, it is a point on which he has arrived at no very satisfactory conclusion.

As the pollen tube apparatus continues to grow the blepharoplasts also increase in size, and about the first of April the contents, which stain red with safranin in the Flemming triple process, become more or less vacuolate (fig. 29). This occurs when the blepharoplasts have reached about half the diameter which they finally attain. During the general increase in size the kinoplasmic rays have become more abundant and in many instances may be seen running from the blepharoplasts out to the plasma membrane of the cell with which they seem to connect.

GROWTH OF BASAL END OF POLLEN TUBE.

The entire prothallial apparatus continues to increase in size until about the middle of May in *Z. floridana* and the first of June in *Z. pumila*, when the central cell and blepharoplasts have reached their full size and the preparation for their division begins. The pollen tube, which has been gradually increasing in length and diameter, has now reached the extent of its growth in the tissue of the nucellus and has become more or less gorged with starch and reserve food materials. The tubes, of which there are commonly from 4 to 8 and sometimes as high as 13 or 14 in a single nucellus, usually grow to a length of 2½ to 4 mm. and are from 80 to 150 μ in diameter in the tissue of the nucellus. They are ordinarily unbranched, but occasionally a branched tube is observed. The writer has never observed an instance where a tube had branched more than once. At this period of development, and later until fecundation takes place, the pollen tubes can be plainly seen in an examination of the apex of the nucellus with the unaided eye. The tissue next to the tubes is brownish or yellowish, clearly marking the path of the tube. Furthermore, the tube usually causes

a protrusion of the nucellar tissue over it, which makes its course easier to follow. It is particularly interesting to note that when several tubes develop in the same nucellus they apparently do not grow altogether at random, but divide the space almost equally, radiating out from the pollen chamber as a center, with almost equal angles between them. There is no special structure of the nucellar tissue which guides this distribution of the tubes, so far as can be observed, and the pollen grains are apparently not guided in their distribution or position in the pollen chamber. When a number of grains are found in the same nucellus they may be scattered in the lower part of the pollen chamber or grouped more or less together. When they germinate, and the tubes turn toward and enter the nucellar tissue, two or more may be found to enter the tissue and start in the same direction. As they elongate, however, they diverge and divide the space between them, and this is a very necessary provision, apparently, as they are so large that in the thin nucellus of old stages there would not be room for them to grow together and cross. The writer has examined several thousand ovules in condition to show this feature well, and in no instance have two tubes been found very close together. In case there are many tubes they are necessarily nearer together than when there are only a few tubes. If there are only two or three tubes they may not divide the space exactly equally between themselves, but are invariably found to be separated by a fairly wide angle. In no case have the tubes been found bunched together on one side of the nucellus, as would occasionally occur if there were not some force guiding their direction of growth. It seems probable that when a tube enters the nucellar tissue it may produce a chemical change of some sort which serves to repel other tubes. It may, on the other hand, be simply a reaction to richness of food supply. Their distribution and growth in the nucellar tissue seem similar to that of root distribution in the soil, exclusive of the factor of light.

During the growth of the pollen tubes the nucellus has continued to change considerably in form and shape. In the early stages of growth the apex of the nucellus has a considerable thickness compared with the pollen tubes (fig. 5). The tubes at first grow straight out laterally in the apical tissue of the nucellus (fig. 5), but before reaching the surface they turn downward and continue growing down through the tissue of the sides of the nucellus just underneath the surface (fig. 51). As the development continues the nucellus grows in size, but gradually becomes thinner, until at the time of fecundation it is reduced to a thin, papery membrane, except at the apex, where other changes have meanwhile taken place. The tissue below the apex of the nucellus which was at first pointed becomes more or less contracted and sunken, as shown in figure 51. The tissue below the pollen chamber and between it and the prothallus, which is at first about 2 mm. in thickness, is gradually absorbed and finally entirely breaks away, leaving an opening from

the pollen chamber into the archegonial chamber. The pollen tubes in early stages grow straight out laterally, the pollen grains being frequently above (toward the apex of the tube), so that the tube in germination at first pointed downward and then curved to one side in entering the tissue (see two lower tubes in fig. 5). Such tubes as these, and also those which develop in other directions, become reflexed during the changes which occur in the apex of the nucellus, and finally the proximal ends of all tubes (the pollen-grain end) are found to be turned down so that they point toward the prothallus. A short time before fecundation, active growth begins in this end of the tube. The proximal ends of the tubes elongate and push down in the pollen chamber farther and farther, the cavity becoming larger and changed in shape. Finally the tissue of the nucellus below the pollen chamber breaks away and the pollen tubes hang down in a cluster in the archegonial chamber. They continue to grow in length until, at the time they burst and discharge the spermatozoids in the process of fecundation, the proximal ends, which hang down free from the tissue (fig. 51), have reached a length of from 1 to 2 mm. During this growth and up to the time the tube bursts the old pollen grain forms a little protruding end at the tip of the tube, which remains covered by the exine of the pollen grain. It stains red with safranin in the Flemming triple process, and is thus easily differentiated.

An extremely interesting fact in connection with the pollen-tube development is the migration of the pollen-tube nucleus, which takes place apparently at the beginning of the active growth and extension of the proximal end of the tube. It will be remembered that when a pollen grain germinates the tube nucleus passes out of the pollen grain and takes a position near the end of the growing pollen tube, and during the entire active period of growth of this end of the tube remains relatively in this position, passing farther into the tube as the latter extends in length. In the course of the investigations it was found that when the active development had begun in the basal end of the tube, a nucleus corresponding in size and appearance to the tube nucleus was almost invariably found somewhere near the central cell. At first it was thought that this was an abnormal occurrence, the pollen-tube nucleus having remained attached to the prothallus in some way and failing to migrate into the pollen tube, as it developed, in the normal way. Investigations showed that this nucleus, however, is normally found near the central cell in this stage of development. It would seem, therefore, that either it must be the pollen-tube nucleus which has migrated from the apical end of the tube to this location or that the pollen tube nucleus divides at some time during the development of the tube, and that one of the two nuclei remains in this location. Juranyi (72) found that in the pollen tubes of *Macrozamia*, grown in artificial cultures on piece [illegible]uit, the pollen tube nucleus after a time often div[illegible]e appar-

ently did not observe the process of division leading to the formation of the second nucleus, and Strasburger was unable to confirm this portion of Juranyi's observation.[1] Recently, however, several investigators have found the pollen-tube nucleus in various other plants to divide, at least occasionally. This was first observed by Chamberlain in *Lilium philadelphicum* (19), and later by Fullmer (42) in *Hemerocallis fulva*. A somewhat careful search was therefore made in various stages of developing pollen tubes of *Zamia* for a division of the pollen-tube nucleus, but no indication of such a division has ever been observed. Furthermore, in an examination of the tubes where the nucleus is found near the central cell no case has been found where a second nucleus occurs at the apex of the tube. It would thus seem that the tube nucleus remains at the apex of the tube as long as this is the end where the most active growth is taking place, and then, when the active growth begins at the base of the tube in its elongation preceding fecundation, it migrates to that end of the tube in order to be near the point of greatest activity and superintend the growth of the pollen-tube wall in this location. Haberland (50), in his extensive paper on the relationship between the function and position of the cell nucleus in plants, has shown that the nucleus ordinarily takes position in the cell near the point of most active growth. The writer has come to the conclusion that in *Zamia* the pollen-tube nucleus remains normally near the distal end of the tube as long as the tube is growing in length and absorbing nutrition. The growth of the basal end of the tube does not start, apparently, until the tube has attained its full development in length in the apical portion. The tube nucleus then migrates to the base of the tube, the increased length of that end of the tube being due to the growth of the pollen tube in that region, thus necessitating the presence of the nucleus. This migration of the pollen-tube nucleus was not discovered until the writer was closing his investigations, and has not been as thoroughly investigated as the other processes of development described. He feels, however, that there is but little doubt of the correctness of the interpretation. Since this conclusion was reached the writer finds that the same migration of the tube nucleus was observed by Ikeno (69, p. 573).

> In the middle of September the embryonal nucleus [tube nucleus] begins to move forward toward the body cell (fig. 22a, Pl. IX), and by the end of the same month it comes in contact with its posterior end, so that at this time the body cell and the outer prothallial cell, as well as the embryo-cell and stalk-cell nuclei, meet at the terminating exine end of the pollen tube.[2]

[1] Ebensowenig trat über geprüften Falle der Pollenschlauchkern auch nur ein einziges Mal in Theilung ein. (Strasburger, 109, p. 3.)

[2] Mitte September beginnt der Embryonalzellkern [tube nucleus] nach der Körperzelle sich hinzubewegen (fig. 22a, Taf. IX) und Ende desselben Monats kommt er in Contact mit ihrem hinteren Ende, so dass zu dieser Zeit sowohl die Körperzelle und die äussere Prothalliumzelle, als auch der Embryonalzell- und der Stielzellkern an dem mit der Exine abschliessenden Ende des Pollenschlauches zusammentreffen.

Hirase (61) has also found that there is a similar return of the tube nucleus to the pollen-grain end of the tube in *Ginkgo*, so that the process would seem to be a general one in cases where this type of fecundation occurs.

During the downward extension of the basal end of the pollen tube the prothallial apparatus remains attached to the base of the tube and is carried down with the tube in its elongation. The central cell in the course of this development is frequently compressed and drawn out so that its shape is greatly altered (figs. 48, 49, and 51). In stages preceding this development the central cell is normally elliptical or oblong, its major axis corresponding to the major axis of the pollen tube (fig. 20). After the tube has turned downward and is sufficiently developed so that the basal end is free from surrounding tissue, the central cell rounds up and becomes nearly spherical.

In the course of this development and change of the basal end of the pollen tube and the prothallus, the blepharoplasts have also changed their position. Previous to this development they occupied the poles of the nucleus, a line passing through them corresponding to the major axis of the cell and longitudinal axis of the pollen tube. During the growth of the basal end of the pollen tube and consequent change of the prothallus they have changed their position in regard to the pollen tube and nucleus and have come to lie at opposite points on the equator of the nucleus transverse in the pollen tube (as shown in figure 21, though this is after the division of the cell). The writer has not been able to determine whether this change in position of the blepharoplast is due to a definite motion of the blepharoplasts themselves or to a change of shape of the cell and nucleus. It would seem, however, that the blepharoplasts must move in the cell independent of the motion of any other organ. As the central cell is pulled down by the growth of the pollen tube it is not infrequent to find the two blepharoplasts preceding the nucleus. However, in the course of this transformation the blepharoplasts may be found in almost any position in the central cell, but usually remain nearly on opposite sides of the nucleus.

The entire prothallial apparatus continues to increase in size until the latter part of May in *Z. floridana* and about June 10 in *Z. pumila*, when the central cell and blepharoplasts have reached their full size and the division of the central cell begins. In this stage of development all cells of the prothallus retain their relative positions with reference to each other, but the base of the pollen tube in which the prothallus is located has in most instances begun to grow and change its position as described above, and a change in the shape of the central cell has frequently resulted before this stage is reached. The first prothallial cell and stalk cell in this stage retain the same position as described in the preceding stage, but have greatly increased in size (fig. 21).

Both the first prothallial cell and the stalk cell have become filled with numerous starch grains, which are frequently compound (fig. 21), and a cross section of the tube through these cells at this stage (fig. 23) presents a puzzling structure until the method of development of the interior first prothallial cell is understood. Even in this greatly enlarged mature stage the original end of the attachment of the first prothallial cell with the old pollen grain remains the same size as in the early stages (compare figs. 20 and 21), and the point of attachment of the plasma membrane of the second prothallial cell (now a part of the plasma membrane of the stalk cell) is the same distance from the point of attachment to the pollen tube as in the early stages (fig. 21).

The central cell, which just after its formation by the division of the second prothallial cell was about 16.91 μ long by 15.57 μ wide, and which at the time the blepharoplasts appeared had reached a diameter of about 36 μ, now commonly measures 170 μ in width by 190 μ in length. Its size, however, is very variable. The blepharoplasts in which greatest interest centers have also increased greatly in size, in this stage measuring from 18 to 20 μ in diameter. In many instances, probably in the majority of cases at this stage, they have become somewhat compressed at the poles, so that they are more or less elliptical in equatorial section (fig. 59) and round in polar view (fig. 60). They still retain their positions at the poles of the nucleus, lying free in the cytoplasm, in this stage usually rather nearer the surface of the cell than the nucleus, not infrequently being almost in contact with the plasma membrane, which is frequently somewhat indented immediately above them. In the course of the development the contents of the blepharoplasts, which was at first homogeneous and then slightly vacuolate, has become filled with vacuoles which present a beautiful, regular form. A few highly refractive bodies, presenting the appearance of crystals or crystalloids of some sort, are also frequently observed in the blepharoplasts at this stage (fig. 29), but the writer has been unable to learn anything as to their nature or function. In this stage the kinoplasmic radiations have become very numerous and extensive, and are more slender than in earlier stages. They radiate in all directions from the blepharoplast, but are naturally more strongly developed and longer on the sides than above toward the surface of the cell or below toward the surface of the nucleus. The writer has tried to determine how the increase in number of radiations is brought about, but has been unable to solve the problem. He has found no evidence, however, favoring their increase by division, as claimed by some investigators. The cytoplasm of the central cell in this stage has become much more dense than in the preceding stage described, but still presents a beautiful reticular structure in well-stained sections. The distinction between kinoplasm and trophoplasm here is not well marked; indeed, in no place in the development of

the central cell is this distinction evident. In the Flemming triple stain the fibers radiating from the blepharoplast are stained deep purple with the gentian violet as normally occurs, but the reticulum of the trophoplasm also stains the same color, though slightly lighter, and in no case has the stain showed any characteristic differentiation between them. The nucleus of the central cell in this resting stage just preceding division is in most cases strongly indented on each side just below the blepharoplast (fig. 59). It might be assumed at first that this was due to the growth toward the nucleus of the kinoplasmic filaments in an early stage of spindle formation, but careful examination plainly shows that this is not the case. The kinoplasmic rays do not crowd down against the nucleus to any extent, and the spindle is entirely intranuclear when first formed, having no connection with the blepharoplast.

DIVISION OF THE CENTRAL CELL.

The earliest stage in the division of the central cell which the writer has been able to detect occurs in the nucleus, in which here and there an accumulation of highly staining granules takes place, forming small, irregularly arranged groups (fig. 27). The blepharoplasts and other organs of the cell in this stage remain comparatively the same as in the preceding stage. The condensation of chromatin matter evidently continues, and gradually the complete continuous chromatin skein is organized. In the skein stage there seems to be nothing particularly different from the process ordinarily observed in the skein stage of other cells and plants. The chromatin band forms a loose, open coil (fig. 28), occupying but a small part of the nucleus. The small amount of chromatin visible here and in later stages seems out of proportion to the enormous size of the nucleus. Occasionally light lines can be observed radiating from portions of the skein in this stage, possibly foreshadowing the formation of the spindle. This would not seem to be the case, however, as similar lines are also observable in some instances radiating from the groups of chromatin granules in the earliest stage of division. In the skein stage the blepharoplast remains in apparently the same condition as in the preceding stage unless somewhat larger. The kinoplasmic radiations still appear very abundant. This collection of the stainable matters into groups of granules and then into a continuous skein evidently foreshadows a general contraction of the chromatin matters into an irregular mass surrounding the nucleolus, a contracted condition apparently the same as the synapsis condition or stage which occurs normally in the reducing division in the formation of the pollen grains of various plants. Its occurrence in this stage of the development of the spermatozoids or germ cells of *Zamia* is thus of particular interest. This contracted condition of the nucleus in *Zamia* has been observed in a number of instances in some of the very best fixed and

stained sections, and the writer can hardly believe it to be due to contraction caused by reagents, as might be supposed. Indeed, the phenomena shown in *Zamia* would appear to demonstrate clearly that it is of normal occurrence here; for, as shown later, after this collection of the stainable granules in one portion of the nucleus, the other portion of the nucleus remains occupied by a plain reticulum of unstainable matter filled with minute refractive granules. Sargent (96) was able to observe this stage in living nuclei of the pollen grains of *Lilium martagon*, showing that in that instance the apparent contraction phenomenon was evidently a normal one. No conclusive evidence has been obtained, however, which shows its significance in the process of division. In the central cell of *Zamia* it is only occasionally that nuclei can be found in this condition, and it is not a favorable place to study the phenomenon. The collection of deeply staining granules in irregular masses would seem to be immediately followed by their gradually moving toward one side of the nucleus and collecting around the nucleolus. The nucleolus is always in the midst of the mass of granules after the completion of the contraction, and it may be that the contraction is always toward that side of the nucleus to which the nucleolus lies nearest. The conviction can hardly be avoided that the nucleolus must be connected in some important way with the collection of these granules around it. In *Zamia* the early stages at least of this phenomenon would seem not to be a contraction, but rather a movement of the stainable elements, granules, etc., of the nucleus to a region in close proximity to the nucleolus, while a colorless, slightly granular, nonstainable matter remains in the original position. This unstained plasma is clearly visible and unmistakable and retains the original reticular structure (fig. 30). It might at first be assumed that this colorless network was due to the albumen used in attaching the sections, or to some deposit from the paraffin or killing reagents. Such could not be the case, however, as in some places where the nuclear membrane had become contracted away from the cytoplasm no such structure intervened, which would have been the case had it been caused by the albumen cement or any of the other reagents used in the process of killing, imbedding, etc. By a careful examination of the edges of the dense mass of granules occasionally a place may be observed where a few of the stainable granules may be found extending out into a strand of this unstainable matter (fig. 30). It would seem from this that the chromatin granules follow along the reticulum of this hyaline plasm in the process of collecting in the synapsis stage, and that in such cases as the above a few isolated chromatin granules had not yet united with the general mass when the material from which the section was taken was killed and fixed. The reticulum of this colorless plasm seems thus to be coextensive with the reticulum of the densely staining portions of the nucleus, and apparently occupies

its place throughout the nucleus, while the chromatin and linin elements are concerned in the formation of the spiral band. This hyaline plasm may probably be considered the nuclear sap or hyaloplasm which forms the ground substance of the nucleus. But it is certainly arranged in a reticulum, while the hyaloplasm or nuclear sap might be expected to fill the entire space. The chromatin and linin elements alone seem to be directly concerned in the formation of the spirem stage which follows, though this can only be conjectured. The granules which are observable in the stainable portion of the nucleus while in this condition are of two kinds. The larger ones, which are round or elliptical in form and quite regular in outline, stain red with safranin in the Flemming triple-stain process, and have the same structural appearance as nucleolar matter, and, as they disappear later in the development of the chromatin spirem, it is probable that they are some form of reserve food matter which is used up in the further process of development. The other granules stain a deep purple-like chromatin, and are probably of this nature, as they appear to form a part of the chromatin spirem, which can be observed in process of formation in the synapsis condition. By a careful study of the coarsely granular stainable mass of the nucleus in this stage it can be discerned, particularly in the outer portion of the mass, that the smaller protein granules are arranged in chains, which are contorted and tangled together so closely that the arrangement can not easily be made out.

The synapsis condition of the nucleus has been observed by numerous investigators, among them Strasburger (108, figs. 3, 66), Farmer (33, p. 473), Calkins (18, p 105, fig. 3), Sargent (96), Duggar (29, p. 82), Davis (26, p. 96), etc. Its occurrence is now so widely known that there would seem to be no doubt that it is a perfectly normal stage in certain nuclear divisions. In this stage (fig. 30) the blepharoplast, which has become elliptical in most cases, remains apparently unchanged. The radiations of kinoplasm are very abundant, as in preceding stages. The cytoplasm presents a fine reticular structure, the kinoplasmic threads corresponding with the network of the cytoplasm, being nearly straight in the vicinity of the blepharoplast and more or less waved after receding some distance from it. Scattered here and there in the cytoplasm are numerous perfectly round globules, staining exactly the same as the rest of the network of cytoplasm, but seeming to lie between the meshes of the reticulum. They would seem to be excretionary granules of some sort (metaplasm), but their origin and nature have not been followed out.

The above sequence of stages in the prophases of division seem to the writer to be the most probable interpretation, but he feels that the matter is still in some doubt.

The next stage in the division which the writer has been able to observe is when the nucleus is in one of the closing prophases of

division approaching metaphase and the spindle is mainly formed. It is highly important to understand the details of spindle formation, because further light might be thrown on the nature of the blepharoplast, but the writer has not been able as yet to find the necessary intermediate stages to make the process clear.

In the next stage which has been observed the spindle is in an advanced stage of formation and the chromatin matter has become contracted to the center of the nucleus (fig. 31). The nuclear membrane is still intact throughout, the spindle being entirely intranuclear. The spindle fibers which are plainly visible do not as usual draw together at the poles and form a sharp-pointed spindle, but in this stage form a blunt-poled or barrel-shaped spindle, something similar to that described by Fairchild in Basidiobolus (31, figs. 3 to 6). Certain groups of fibers can be seen to be attached to certain chromosomes and extend in a well-differentiated bundle toward the pole, reaching in this stage to the nuclear membrane, which is still apparently intact throughout, being as plainly visible at the poles as in any other region. This would point to the nuclear origin of the spindle, though it is of course possible that the kinoplasmic filaments surrounding the blepharoplast could have penetrated into the nucleus and served to form the spindle. This, however, is not thought to be the case, although it may be remarked that the radiations from the blepharoplast, which were so striking in the early stages of development, are not nearly so prominent in this stage. The intranuclear origin of the spindle, while not of common occurrence, is nevertheless found in some instances among plants, as in the case of *Valonia*, described by Fairchild (30, p. 336), and *Ascophyllum*, described by Farmer and Williams. The latter authors say:

> An interesting feature presented by the achromatic spindle in this, and especially also in the following oogonial divisions, as well as in the divisions of the oospore, lies in the fact that it is largely intranuclear. It begins to be formed before the nuclear wall can be seen to be broken down at the two ends * * * (37, p. 625).

In this stage of the division of the central cell in *Zamia* the nucleolus has already entirely disappeared. The nucleus has the shape of a biconvex lens, being almost elliptical in section, its minor axis corresponding usually to the major axis of the cell (fig. 31). The spindle does not occupy the entire nucleus, and at each side the protoplasm retains the reticular form similar to its structure before the spindle began to form. The most noteworthy variation in structure occurring in the cell at this stage of the division is in the blepharoplast, which has undergone a striking change since the last stage. It has increased in size somewhat, and the outer membrane has separated from the contents, which in the meantime has shrunken somewhat, though not very markedly as yet. The outer membrane of the blepharoplast stains more deeply purple with Flemming triple stain, and has sepa-

rated into fragments or plates, a cross section in this stage showing a broken line. The separations in this stage, however, are yet quite irregular and infrequent. The content of the blepharoplast has contracted considerably, but still retains its vacuolate appearance (fig. 32). The kinoplasmic radiations which in the preceding stage were very strongly developed evidently almost disappeared at this stage, as several well-stained sections of nuclei in this stage show at best only slight suggestions of radiations. The reticulum of the protoplasm is so arranged as to give the suggestion of radiations immediately adjoining the blepharoplast. What becomes of the radiations the writer is unable to say. They may in some way aid in the spindle formation, but the nuclear membrane still remains unbroken. Wilson says that "it is now generally agreed with Van Benedin that the mantle fibers are essentially a part of the asters—i. e., are simply those astral rays that come into connection with the chromosomes" (130, p. 79). This is certainly not the case in *Zamia*, unless we can imagine the rays of the blepharoplast swinging around, losing their connection with the blepharoplast, and penetrating into the nucleus through a well-formed nuclear membrane. The mantle and interzonal fibers of the spindle are both formed and the spindle apparently fully developed before the nuclear membrane breaks down.

As the division progresses and reaches about the metaphase the outer membrane of the blepharoplast becomes more plainly segmented (fig. 61, photograph) and the vacuolated content has become more shrunken and is plainly disappearing. During the metaphase, or slightly before or after, the nuclear membrane breaks up and would appear to become transformed into spindle fibers, which remain in the position previously occupied by the nuclear membrane and preserve the outline of the nucleus.

In an early anaphase of division (figs. 33 and 62) the dividing nucleus presents a perfectly normal appearance so far as the main features of division are concerned. The chromosomes have just pulled apart and are approaching their respective poles; the nuclear membrane has disappeared, but the outline of the nucleus is still preserved by fibers which seem to have been formed by the disorganization of the nuclear membrane. The disappearance of the nuclear membrane seems to be gradually accomplished by its breaking down and becoming directly transformed into fibers of the spindle, outside of the mantle fibers, which spread out in each direction toward the periphery of the cell and later take part in the formation of the new delimiting plasma membrane. A layer of cytoplasm around the nucleus and a hemispherical mass of cytoplasm at each pole presents a different structure and staining capacity from the general mass of the cytoplasm. It is composed of a more open reticulum, which does not stain so deeply as the more dense outside portions. The poles of the spindle end in this

open cytoplasmic mass, and it is in these light polar areas that the daughter nuclei are finally organized. The blepharoplasts lie entirely outside of these light areas a considerable distance from the pole of the spindle. This can be observed more clearly by examining figure 34, which is an enlarged section taken out of cell photographed in figure 62. It will be noticed here that the spindle fibers come to a focus in the lower part of the figure. If now a centrosome or centrosphere of an ordinary kind was present it should be located where the spindle fibers come to a focus. This location, however, is occupied by no body which could be considered to be a centrosome. A most careful examination of the protoplasmic structure has been made at this stage of division and no connection can be discovered between the spindle and the blepharoplast. The radiations from the blepharoplast are, as in the preceding stage, rather inconspicuous and seem to be merely due to the arrangement of the reticulum. Between the ends of such radiations as can be observed and the pole of the spindle lies the hemispherical mass of polar cytoplasm, in which the reticulum presents a totally different appearance from that of the other cytoplasmic areas. The protoplasm here is made up of a loose, open reticulum, in which individual threads may be frequently observed to run for a considerable distance in the same plane. The spindle fibers run into this mass and end rather abruptly, not extending up into it. The spindle fibers can not be confounded with the fibers of the reticulum. There can be no doubt that no fibers run from the spindle to the blepharoplast in any sense in which the spindle fibers focus on a centrosome or centrosphere when such an organ is present. The spindle formation and structure were not investigated in much detail by Ikeno and Hirase; but this same peculiarity of structure would seem to be present also in the plants they studied. Hirase's figure 18 (62) clearly shows that the radiations from the blepharoplast do not connect with those from the spindle, and the pole of the spindle is illustrated as having an entirely separate aster, in the center of which a centrosome should be located if any such organ is present. This is not so clearly shown by Ikeno's figure 3, but in his figure 25a (70), where the nucleus is in an anaphase of division, the blepharoplast is very distant from the pole of the spindle.

The structure of the blepharoplast of *Zamia* in this anaphase is also particularly interesting. The outer membrane is still observed to be split up into a number of segments. The membrane itself in cross section when very carefully examined seems to be made up of numerous granules of comparatively the same diameter placed side by side and making up the membrane. This structure is particularly interesting in connection with what follows when the membrane is broken up and appears merely as a group of numerous granules.

The contents of the blepharoplast, so far as the stainable matter is

concerned, has entirely disappeared, and inside of the blepharoplast now a clear hyaline plasm is visible, forming a delicate reticulum with large meshes somewhat similar to the plasm found in the colorless parts of the nucleus in the synapsis stage of division. What has become of the dense stainable matter which previously occupied the center of the blepharoplast? It will be remembered that this matter appeared largely like nucleolar matter, remaining homogeneous but vacuolate, and staining deep red with safranin. Its contraction away from the outer membrane of the blepharoplast during division and its gradual disappearance as above described indicates that it may have been utilized as reserve food material to provide for the active growth which has been taking place in the blepharoplast itself and in other parts of the cell. The growth of the outer membrane of the blepharoplast in thickness and the appearance of granules in the structure of the membrane is evidently correlated in some way with the disappearance of its contents. When the daughter nuclei organize in an early telophase, they are at first strikingly small in comparison with the organizing daughter cells and the nucleus of the mother cell. Figure 35 shows a drawing of a fairly early telophase, and a photomicrograph of the same cell is shown in figure 63. The nuclei of the daughter cells in this case have reached the daughter spirem stage, the chromatin spiral being plainly visible in one of the nuclei. The nuclei are elliptical in shape, being from 20 to 22 μ long and from 12 to 13 μ wide. The daughter nuclei are located in a portion of the cytoplasm, having a different reticular structure, which evidently corresponds to the specialized polar areas of cytoplasm described in the preceding stage. The spindle fibers have bulged out on each side of the old mother-cell nucleus, reaching the cell wall on each side. They have contracted away from the daughter nuclei, and seem at their outer ends to be in the process of gradual transformation into the normal reticulum of the cytoplasm. No visible thickening occurs on the fibers where the new plasma membrane is to be formed, but a space free of granules and stainable matter clearly shows where the new plasma membrane delimiting the two cells will form. This is particularly well shown in a photomicrograph of the cell shown in figure 63.

The blepharoplast, which in the preceding stage was separating into plates or fragments and showed itself in cross section to be made up of numerous small granules so placed together as to form a membrane, is in this stage represented by a group of numerous round or oblong granules clustered together in a somewhat irregular, more or less spherical mass, which stains the same as the outer membrane of the blepharoplast. It would seem that the outer membrane of the blepharoplast breaks up into numerous segments or granules, which assume a roundish or elliptical form and through the action of the cytoplasm become crowded together in a mass occupying the position

of the original blepharoplast. In the cell shown in figure 35 the granules are from one-half to 1 μ in diameter and stain deep purple with the Flemming triple stain, as does the nuclear membrane. The mass of granules here occupies more space than the daughter nuclei and form a somewhat rectangular mass from 10 to 13 μ wide by from 20 to 22 μ long. The cell figured here is smaller than usual, being only 132 by 174 μ. The radiations from the blepharoplast would seem to be simply accentuated strands of the reticulum, the radiations running irregularly and corresponding with the walls of the cytoplasmic meshes. The radiations are by no means plain in this stage, and end rather indefinitely when they approach the granules of the blepharoplast. There is no indication of any membrane surrounding the blepharoplast in this stage.

In a late telophase, when the daughter nuclei approach a resting condition they are found to have greatly increased in size, while the group of granules remains of comparatively the same size, and to all appearances unchanged. In this closing stage of the division, however, a most wonderful process is just starting the organization of the cilia-bearing band of the spermatozoid, which is formed by the union of the granules of the blepharoplast. At first the band can be detected only as a delicate, short, deeply stainable line, extending from the group of granules of the blepharoplast toward the nucleus. At first apparently only one of these lines can be observed, but shortly, as development progresses, a similar line can be observed protruding from the mass of granules on the opposite side (fig. 39). At first the band is very narrow, being scarcely more than a line. It gradually increases in width, however, as it increases in length, till it soon has an appreciable width. While there would seem to be no possible doubt that the band is organized at the expense of and by the granules of the blepharoplast, the details of the process of the organization is somewhat difficult to discover. In some sections the writer has been able to distinguish what seems to be individual granules of the blepharoplast fusing with the band where it joins the mass, several of the granules that have united with the band still showing their individuality (figs. 36 and 37). Again, in bands which have developed considerably the thickness seems to be continually added to by the fusion of other granules with it directly on the edge which lies next to the mass of granules (figs. 38 and 64). It is not infrequent to find sections showing indications of this sort, which suggest the union of the granules together to form the band. Furthermore, as the band grows in length the granules of the blepharoplast gradually disappear till all have been absorbed in the growing band. The writer thus feels that there is little doubt of the correctness of the interpretation. This history of the origin of the cilia-bearing band by the fusion of the granules of the blepharoplast was first described

by the writer at the August 22, 1898, meeting of the American Association for the Advancement of Science, and was published in Science, November 11, 1898 (127). The same conclusion as to the origin of the cilia-bearing band in *Cycas* was reached by Ikeno apparently about the same time, and published in his monograph which appeared somewhat later, November 23, 1898 (70, p. 575). The beak which extends out from the nucleus to the blepharoplast and forming band in *Cycas*, as described by Ikeno in *Cycas* (70) and by Hirase in *Ginkgo* (62), the writer has not been able to find in *Zamia*, even after renewed search. There would seem to be no reason, however, to doubt the correctness of the observations of Ikeno and Hirase. It may be that such a nuclear beak is formed also in *Zamia*, but was contracted in the writer's specimens by the reagents. There is nothing to indicate this, however, and the writer believes that no such nuclear extension or beak is formed in *Zamia*. How it could have escaped observation in the long-continued researches of the writer when special attention was given to searching for it is difficult to imagine. Similar beak-like extensions of the nucleus toward blepharoplasts have been observed by Strasburger (112) in swarm spore formation, and toward the centrosphere by Harper in the formation of the ascospore walls in *Erysiphe* (51, figs. 18–25) and *Lachnea* (52, figs. 43–45). In a late stage of the spermatozoid formation in *Zamia*, when the ciliferous band has completed its growth, numerous little points have been observed extending out toward it from the nucleus, but these extrusions are certainly not connected in any intimate way with the process of band formation.

In the stage when the ciliferous band first begins to organize, the daughter nuclei are usually approaching a resting stage (fig. 36) and one nucleolus and sometimes two have already been organized in each. The spindle fibers have shortened up materially and the cell plate is usually fairly well organized. The formation of the plasma membranes separating the two cells is frequently shown with great clearness in *Zamia* and one can observe all stages of the transformation of the spindle fibers into the reticulum on one side and the plasma membrane on the other. The spindle fibers gradually shorten in length and contract into the cell plate, apparently forming the material for the organization of the separating membranes. Even in an early stage of the organization of the separating membrane, when only a few meshes of the cytoplasmic recticulum intervene between the daughter nuclei and the ends of the spindle fibers, the place where the future cell wall will form is plainly shown by a slightly lighter, clearer area crossing the cell (figs. 35 and 63). As the cell advances thickenings appear on the spindle fibers where the new wall is to form. The fibers continue to contract more and more until they become very short. A lighter staining mass of protoplasm then shows on each side of the forming

wall into which the short ends of the spindle fibers extend, and in which their gradual transformation into the ordinary reticular structure of the protoplasm seems to be taking place (fig. 44). Ordinarily the double plasma membrane can not be distinguished readily, but in some instances it is plainly discernible, being due evidently to slight contraction. In the case of the division of the central cell which gives rise to the spermatids no cellulose well is formed between the cells. In cases where such a wall is formed or normally occurs it would doubtless be laid down between the two plasma membranes. As to the question of the division of the swellings on the spindle fiber into two portions in the formation of a double plasma membrane no evidence has been obtained. The two membranes normally appear as a single membrane and apparently, unless there is some contraction, the presence of a double membrane can not be determined. The details of the origin of the plasma membrane here correspond in general, so far as traced out, with the conclusions of Strasburger. A double plasma membrane, such as is seen in the central cell of *Zamia*, is also described by Blackman (16, p. 480) in the formation of the ventral canal cell in *Pinus*.

In a stage slightly later than the one above described the division may be considered as completed; the daughter nuclei having apparently returned to the resting condition (fig. 36). The organization of the cilia-bearing band, however, is yet incomplete. The band and blepharoplast granules still lie free in the cytoplasm, about midway between the nucleus and the plasma membrane. The band by this time has grown to an appreciable thickness and increased greatly in length. It now forms a crescent-shaped body with the granules grouped on one side (figs. 38 and 64). The band in this stage is usually about 7 to 10 μ in width at broadest point.

METAMORPHOSIS OF THE SPERMATIDS.

After careful consideration the writer has concluded to adopt the zoological term *spermatids* in designating those cells which become metamorphosed directly into spermatozoids. The change in the daughter cells from the closing of the division of the central cell up to the maturing of the spermatozoids is thus discussed under the heading *Metamorphosis of the Spermatids*. The nomenclature here used corresponds with that used by Shaw (102) in *Marsilia*. The use of *spermatid* and *spermatozoid* instead of *antheroblast* and *antherozoid*, as the writer at first intended, seems commendable as terms having a corresponding meaning in zoology and thus more generally understood. The central cell and intermediate cell generations can not be given terms similar to the zoological ones, as the process of development in plants is very different from that of animals, the re

division occurring in the division of the pollen mother cell preceding the division which gives rise to the pollen grains.

The most marked change takes place first in the ciliferous band, which continues to grow in length and width, and develops kinoplasmic radiations from the outer surface. These radiations sometimes become very prominent, reaching out nearly to the plasma membrane (fig. 41). In the majority of the writer's sections, however, no prominent radiations like these can be discovered. It is not long, however, till small papillæ are formed on the outer surface of the band which evidently develop into cilia later (fig. 40). It may be that these protuberances occur while the band is young and grow into the marked radiations later. However this may be, it is certain that in a later stage the radiations disappear again or are contracted to small protuberances on the outer surface of the band. The band up to this time lies free in the cytoplasm of the cell about midway between the nucleus and surface.

The minute structure of the band in the spermatid in *Zamia* reminds one forcibly of the structure of the tail of certain animal spermatozoa. While the band is still short, in fact almost as soon as it can be clearly distinguished to have width, one edge of the band seems to be denser and heavier than the other edge. This is the edge on which the granules unite. In the mature spermatozoid, however, this distinction in the thickness and density of the different edges of the band can not be plainly distinguished.

As the differentiation progresses the band, which has meanwhile absorbed all of the granules of the blepharoplast, becomes greatly extended in length, moves out away from the nucleus and becomes more or less closely applied to the plasma membrane of the spermatid. The band by this time has increased greatly in length and in this stage forms from one to two turns around the cell. In its growth the band is always so extended that it forms a helicoid spiral. The turns, when viewed from the apex, always running in a direction contrary to the hands of a clock, the spiral formed by the growth of the blepharoplast band is thus a levotropic one. When the ciliferous band has completed one turn around the cell it has usually taken a position on the equator of the cell in such a way that it completely encircles the nucleus of the cell. In median sections through a spermatid at this stage the band is seen in sections on opposite sides of the nucleus (fig. 42). If the series of sections of a pair of spermatids is traced through till the upper section is reached the surface of the single band will be found to stretch entirely across it above the nucleus. To reach this location from the point where it was organized by the blepharoplast granules, the band has traveled almost the entire width of the cell. It can be observed in this stage to be in close proximity to the plasma membrane, but evidently not fused with it, as the writer

was at one time inclined to believe. Immediately over the band the plasma membrane of the cell is invariably strongly indented. The cilia which develop from the outer surface of the band in this stage are little more than protuberances which seem to strike the plasma membrane of the cell and finally penetrate it and grow into mature cilia. The band continues to grow in length, but meanwhile decreases somewhat in width. It is broadest near the apical end and decreases in width gradually at both ends. The elongation continues until the band has developed five or six continuous turns around the cell. In this mature condition it is found to have developed in the form of a helicoid spiral with the apex located at the point of the cell corresponding to the original position of the blepharoplast. It is thus opposite the point of contact of the two spermatids in each case. During the growth of the ciliferous band the nucleus of the spermatid has increased in size, growing very markedly at the expense of the cytoplasm. The entire cell increases in size meanwhile, but not so rapidly as the nucleus.

STRUCTURE AND FORM OF THE MATURE SPERMATOZOID.

The mature spermatozoids, before they start to swim, differ but little in appearance from the spermatids in the last stage described (fig. 42). In all cases the two spermatozoids which result from the division of the same central cell remain attached together until active motion starts. When mature, and before separating, each spermatozoid is irregularly hemispherical in shape (fig. 45). The nucleus occupies a large portion of the cell, but is plainly surrounded on all sides by a layer of cytoplasm. The karyoplasm is coarsely reticular and open, and there is almost invariably one or two nucleoli present. The cytoplasm surrounding the nucleus on all sides is very densely granular and stains deep violet blue with the Flemming triple process. It is so densely granular that it is almost impossible to distinguish the reticular structure of the protoplasm. The ciliferous band in the mature spermatozoids is plainly shown, forming a helicoid spiral of from five to six turns which covers about one-half of the body of the spermatozoid. At the apex of the spiral the band apparently leaves the surface of the cell and gradually fades out, the small end occasionally making a complete but inconspicuous turn in the cytoplasm of the cell. At the open end of the spiral the band decreases in width and finally fades out entirely. The band retains the shape described in the older stages of the spermatid, being distinctly broadest near the apical end of the spiral and becoming narrowed to a point at each end. The following is the width of the band at each of the six cross sections on one side of a spermatozoid in a median section, beginning at the apex of the spiral: 5.1 μ, 8.9 μ, 7.7 μ, 5.1 μ, 5.1 μ, 3.8 μ. These figures would correspond closely with those taken from any other full-grown

spermatozoid in section. The plasma membrane over the band is strongly indented both in spermatids before motion has started (fig. 45) and in the spermatozoids swimming free. This forms a deep helicoid furrow on the outside of the spermatozoid body. Below this indentation lies the ciliferous band, frequently in such close connection with the plasma membrane that it is difficult to determine that the band has not fused with it. In some instances, however, in the most mature specimens, it can be seen that the band remains distinct and that the very numerous cilia growing from it penetrate through the plasma membrane (fig. 46).

An interesting question is presented in regard to the structure of the ciliferous band. In some sections it would seem to be made up of numerous fine granules placed together side by side in such a way as to form a connected membrane, and the cilia appear to grow from these granules. It would be interesting to know if these individual granules are the same granules in each case as occurred before the organization of the band when the blepharoplast had broken up into a mass of granules. While this would seem probable and a natural sequence, no direct evidence has been discovered in its support. It might be added that the number of cilia on the band would seem to be larger than the number of granules in the blepharoplast, but no actual estimation of the number of these has been made in either case. The development of the cilia from definite granules in the band can be analogized with the granules which Strasburger found at the base of the cilia in the swarmspores of *Œdogonium Vaucheria*, etc. (112).

Another feature of importance in the spermatozoid formation of *Zamia* is the metamorphosis of the entire spermatid cell into a spermatozoid. In the writer's preliminary papers it was pointed out that when the central cell divides to form the spermatids a plasma membrane is formed entirely across the cell, and that in the transformation of the two daughter cells or spermatids into the spermatozoids these cells are transformed directly into the spermatozoids without the formation of a new plasma membrane. There is thus no formation of the spermatozoids inside of a mother cell and the differentiation of new walls around the spermatozoids, as had been described in all previous cases of spermatozoid formation, so far as known to the writer. The correctness of the writer's observations were questioned by some botanists, and this has led him, in later investigations, to give special attention to this point. Further study, however, has only confirmed the view first stated. The spermatids are made up of the entire daughter cells resulting from the division of the central cell. The correctness of the writer's observations on this point were confirmed by Ikeno in his study of the spermatogenesis of *Cycas* (70), where no mother-cell membrane or wall inclosing the spermatozoids was found. In *Ginkgo* the matter still remains in doubt. Hirase (62) does not dis-

cuss the matter directly, but his figures, 19, 24, 26, and 28, might be taken as indicating an inclosing membrane. Fujii's figures (40, figs. 2 and 5) also represent the formation of the spermatozoids inside of a mother cell, although here again apparently no special attention was given to this point, and the appearances of the figures, as will be shown later, are capable of other explanation. Coulter and Chamberlain have recently called attention to this apparent difference between the development of *Zamia* and *Cycas* and *Ginkgo* (24, p. 44). The same authors draw a distinction between the spermatozoids of *Zamia* and *Cycas* and those of lower plants which the writer thinks can hardly be maintained. They say: "It is these ciliated cells which have been called spermatozoids or antherozoids, and such they are physiologically. Morphologically, however, they are sperm mother cells which do not organize sperms, a fact which seems true of all spermatophytes." The organization of the whole cell into a spermatozoid is considered by them to be very different from what occurs in the Pteridophytes. "The contrast with Pteridophytes, in which each mother cell organizes an internal ciliated sperm and discharges it, is sharp." It is difficult to harmonize this statement with the recent researches of Shaw (102) and Belajeff (12) on *Marsilia*, where it is clearly shown that the spermatozoid is the entire mother cell metamorphosed. It is too early to generalize, but the writer is inclined to the opinion that where spermatozoids are differentiated inside of a mother cell which is discarded it will be found that it is the cellulose shell only, if such is present, which is thrown away. The plasma membrane (*Hautschicht*), from which the cellulose wall is apparently secreted, probably draws away from the worthless cellulose shell in the spermatozoid formation so that the entire cell, morphologically, is utilized. Nothing is lost in nature as a usual thing. How then could we expect the plasma membrane, which is apparently simply a modified form of active kinoplasm, to be thrown away? The fact that a double plasma membrane delimiting the daughter cells is first formed in cell division, each cell having its own membrane, and that if a cellulose wall is formed at all it appears later between these membranes, indicates that the wall is of secondary importance, which is further supported by the fact that in many cell divisions, as in all the prothallial cells of *Zamia* and *Ginkgo*, no cellulose walls are formed, the cells being delimited only by the plasma membranes. The cell wall the writer looks upon as a secretion and not an active organ of the cell, the discarding of which could not be looked upon as indicating a different morphology. In case more than one spermatozoid is formed within a cell their formation must be preceded by karyokinesis, which would doubtless divide the protoplast into as many distinct cells.

Strasburger's recent investigations of swarm-spore formation may be cited in support of the writer's view on this point. He states that

in his earlier investigations on *Vaucheria* he was mistaken in describing the dissolution of the *Hautschicht* of the sporangium and the formation of a new *Hautschicht* around each spore (112, p. 188). In the case of *Œdogonium*, also according to Strasburger, the *Hautschicht* of the sporangium goes to form the *Hautschicht* of the swarm-spore. Strasburger says: "Die Hautschicht des Sporangiums liefert auch hier thätsächlich die Hautschicht der Schwärmspore."

In studying the living pollen tubes in sugar solutions, considerable search has been made for evidence bearing on this point. In no case, however, has a definite membrane connected with the stalk cell been found inclosing the spermatozoids, which could be considered as the wall of the mother cell. When the spermatozoids pull apart, however, an appearance is sometimes observed which might suggest the presence of a mother cell wall. If mature pollen tubes, in which cilia motion has not begun, are placed in sugar solution, the cilia begin to vibrate and the spermatozoids gradually pull apart and round up, as described elsewhere in this paper. When the cilia first begin their motion the surrounding protoplasm seems to hold together and spring back and forth by the beating of the cilia, as if bordered by a definite membrane. When the spermatozoids round up they occupy less space than when they are attached and quiescent. In certain tubes the protoplasm surrounding the spermatozoids holds together tenaciously when the spermatozoids begin motion, strongly suggesting the presence of a mother cell wall. When the spermatozoid strikes against it or when hit by the cilia, the protoplasmic mass does not break up, but shows elasticity, springing in and out with the impinging of the cilia, etc. This is seen to some extent whenever spermatozoids are observed starting their motion, but it is seldom very noticeable. Usually the protoplasm soon breaks up and the spermatozoids swim about unobstructed. While these observations suggest the presence of a mother cell wall, the writer believes that it must be interpreted in another way, as it is certain from a study of prepared sections that no distinct wall from that of the mother cell is formed around the spermatozoids. It would seem that the protoplasmic structure is not easily broken up and hangs together tenaciously in some cases. Again, the plasma membrane of the pollen tube, which is a single cell, surrounds the entire prothallial apparatus, and it is probably this membrane which gives the spermatozoids some difficulty in breaking through into the general protoplasmic contents of the pollen tube. The plasma membrane of the pollen-tube cell is not so easily differentiated as that of the cells of the prothallial apparatus, but is usually clearly distinguishable, and would probably form an obstruction to any object like a spermatozoid entering it. Considering the structure and phenomena presented, the writer has been led to the conclusion that the description of the structure given in his preliminary paper is

correct, and that Hirase and Fujii probably erred in figuring a mother cell in *Ginkgo*, inside of which the spermatozoids are differentiated. It is important to note furthermore that in Fujii's figures (40, figs. 2 and 5), the drawings, when critically examined, do not show the differentiation of the spermatozoids inside of the mother cell, as no wall is shown separating the two spermatozoids. When the central cell divides into two cells they are separated by a definite membrane. If, then, each one of these mother cells develops a spermatozoid internally, this separating membrane should remain between the developing spermatozoids, but this is evidently not the case, judging from the figures. Fujii's figure 2 seems to illustrate exactly the same appearance as that described above as occurring in *Zamia* when the spermatozoids pull away from each other and round up so that they occupy less space, and have their original location marked by the surrounding plasma membrane of the pollen tube. The writer believes that it may be safely concluded that *Ginkgo* corresponds with *Zamia* and *Cycas* in the metamorphosis of the entire cell, and, as stated above, believes that this method of differentiation is in harmony with what is found in *Marsilia*, and probably in other ferns and lower plants.

MOVEMENT OF SPERMATOZOIDS.

For purposes of microscopic study the pollen tubes were cut off some distance above the prothallus and placed on ordinary microscopic slides, hollow-ground slides, or in glass chambers in solutions of cane sugar. In the beginning of the studies water was used, but this proved very unsatisfactory, as the spermatozoids soon died and burst, evidently from the difference in density of water and the contents of the cells. Solutions of cane sugar of several strengths were tried and a solution of about 10 per cent gave in general the best results. By the use of this solution the spermatozoids were kept living and moving for a considerable time, making it possible to study them quite carefully in a living condition. If they are transferred to the sugar solution without injury they usually continue to move from thirty to sixty minutes and one instance was recorded where motion continued for two hours and forty-four minutes. The feat of cutting off the pollen tubes which hang down from the apex of the nucellus, as shown in figure 51, is by no means as difficult as might be supposed, judging from the size of pollen tubes and sperm cells in plants ordinarily. Here the pollen tubes and spermatozoids are so large that they are plainly visible to the unaided eye and can be easily handled under an ordinary dissecting lens. In the manipulation the ovaries are cut open and the upper part of the membraneous nucellus with the pollen tubes removed. The nucellus can then be inverted over the first finger of the left hand and held in place by the thumb and second finger. Held in this way the tubes protrude prominently and can be easily cut off with a sharp

scalpel or razor and transferred to the microscopic slide. In many cases the writer succeeded in cutting off the tubes nicely by placing the tip of the nucellus with the pollen tubes uninjured on the slide in sugar solution under a dissecting microscope and while holding it with forceps severing the tubes by a lateral cut. In this way one may frequently get uninjured spermatozoids swimming free in the solution, and it is then a striking sight to observe them. It is difficult to believe that the little opaque white spheres, which can be seen very easily with the unaided eye and can even be observed to move around, are really spermatozoids. It is not a difficult matter to obtain the spermatozoids moving in *Zamia*, the writer having observed hundreds of them living and moving. He has shown them to many friends, including some twenty of the Washington botanists.

In removing the nearly mature pollen tubes the spermatozoids are found to be in various stages of development, as would be expected. In many cases tubes have been observed, before cutting them off, in which the two spermatozoids had pulled apart and were swimming free in the protoplasm. In some instances their movement in the pollen tube, before it is injured, can be observed with the aid of a hand lens. This was first noticed by my colleague, Dr. Erwin F. Smith, in material which he was looking over with the writer, and was later observed by the writer in many instances.

Evidently this is what occurs normally in the development of the spermatozoids, just before fecundation, as numerous instances have been found in prepared sections of material, just at the time of fecundation, in which the spermatozoids had broken loose from the stalk cell, pulled apart, and were swimming free in the pollen tube (fig. 68). Their lively motion probably has considerable to do with the bursting of the pollen tube when they are discharged in the prothallial cavity over the archegonia in the normal course of fecundation. In many instances tubes which were cut off and placed in sugar solution were much younger and showed the prothallial apparatus entire, exactly as shown in sections. The second prothallial cell inside of the stalk cell in such instances showed very plainly, being of a darker color than the latter. The nuclei in these two cells, however, could not be discerned in the living material, probably owing to the surrounding starch, etc. The two spermatozoids in such tubes remained attached, though apparently matured. When mature pollen tubes of this nature are cut off without injury and placed in sugar solution the prothallial cell, stalk cell, and spermatozoids can at first be seen to have their normal shape. In a few minutes, however, when the sugar has had time to diffuse into the pollen tube the spermatozoids gradually begin to move. A few cilia start the movement, contracting irst; then gradually the other cilia begin to move and tion increases until soon all of the cilia are ey

can hardly be seen. The spermatozoids after starting motion soon break loose from the stalk cell, which quickly collapses. Shortly after this they begin to gradually round up and pull apart, the ciliar motion continuing very active meanwhile. As they are located previous to this, closely pressed together with their major axes crossing the tube (fig. 47a), there is not room for them to separate by pulling directly apart, so they stretch in opposite directions, bending toward the longitudinal axis of the tube (fig. 47 b and c). They continue to round up more and more and finally pull entirely apart (fig. 47d). In one tube of this nature, when first cut off and placed in sugar solution, streaming motion of the protoplasm of the pollen tube was noticed in some strands immediately above the spermatozoids. This was entirely interrupted as soon as the motion of the spermatozoids began.

In the examination of fixed and stained sections of tubes in which the spermatozoids were swimming, there seems to be very little protoplasm in the tube. The living tubes, however, present every evidence of being very turgid and completely filled, and such is doubtless the case. In tubes which are cut off quite long and are uninjured in the transfer, such as the tube mentioned above in which streaming motion was observed, the entire tube is seen to be filled with granular protoplasm, with definite strands occurring here and there. The spermatozoids when they first begin moving have some difficulty in breaking through the plasma membrane of the pollen tube cell and entering the general protoplasm of the pollen tube (see page 53); when this is accomplished, however, they swim back and forth with considerable ease. It is an interesting sight to see the two giant spermatozoids moving around vigorously in the pollen tube, bumping against each other and the wall of the tube in their reckless haste. They seldom escape from the upper cut end of the pollen tube, although they as frequently swim toward this end of the tube as the other end, so far as could be observed. In many cases the pollen tubes were cut so that the spermatozoids escaped into the solution, and in numerous other cases mature turgid tubes burst in the process of cutting, discharging the uninjured spermatozoids in the sugar solution. The writer was thus able in many cases to study the spermatozoids swimming free and observe their unobstructed motion. The plasma membrane of the spermatozoids is very tender, however, and is commonly broken in attempting to remove them from the pollen tube. When swimming free without pressure they are slightly ovate, nearly round or compressed spherical (fig. 52). They vary greatly in size, but are commonly slightly longer than broad, ranging in length from 222 to 332 μ and in width from 222 to 306 μ. The spermatozoids of *Ginkgo* are described by Hirase (62, p. 123) as being egg-shaped and 82 μ long by 49 μ wide. Those of *Cycas* are said by Ikeno (70, p. 580) to be 160 μ long by 70 μ wide. The spermatozoids of *Zamia* are thus much larger than those of *Cycas*

or *Ginkgo*, and so far as the writer has been able to learn are the largest that have been observed in either the animal or vegetable kingdoms. The cilia are also very prominent and numerous, measuring from 40 to 50 μ in length. When observed under the microscope in a living condition the spermatozoids are densely granular and of a yellowish brown color by transmitted light. By reflected light under a low magnification they are white and opaque. The cilia and the helicoid spiral band to which they are attached can be easily seen, while the structure of the nucleus and cytoplasm is so similar that the nucleus can not be discerned.

The motion of the spermatozoids when swimming free in sugar solution is in no way different from their motion when in the pollen tube. The general motion is a continuous rotation of the body, always in the same direction, around an axis passing through the apex of the helicoid spiral. Viewed from the head end or apex of the spiral the rotation is in the direction of the hands of a clock and contrary to the turns of the spiral band. They roll around, first here, then there, resembling in this respect the motion of *Pandorina*. After moving about rapidly for from five to fifteen minutes they usually cease all progressive motion, but continue to rotate for a considerably longer period. The rotary motion also soon ceases, but the cilia continue to vibrate for a considerably longer time. The spermatozoids of *Zamia* also have an amœboid motion, which is particularly noticeable while they are inclosed in the pollen tube. The apex of the spiral as a whole frequently rotates in a most remarkable way, turning in a circle, pushing out first this way and then that way with the greatest freedom of motion, as if selecting a point of exit or ingress. In other cases the base or the side of the spermatozoid body may be considerably extended as a blunt point in pushing between two obstacles. The whole body seems flexible and changeable in the highest degree and is eminently fitted for its difficult task of finding and swimming through the narrow passage between the neck cells of the archegonia. This amœboid motion is highly suggestive in connection with the possible motility of nonciliated sexual cells in sea weeds and seed plants. It seems almost certain that the spiral sperm cells that have recently been described as occurring in a number of seed plants—*Lilium* (Nawaschin, Guignard), *Fritillaria* (Nawaschin), *Triticum* (Goroschankin), *Silphium* (Merrill), etc.—will be found to have such a rotating amœboid motion even if they are not ciliated. The writer is not aware that attention has before been called to this mode of motion in sperm cells.

The vibration of the cilia in vigorous spermatozoids is exceedingly rapid and difficult to study. Judging from observation made on certain spermatozoids just starting motion and others which had nearly exhausted their energy, there would seem to be a rhythmic contraction of the cilia which passes quickly from one end of the band to the

other. A tremulous vibration of the cilia, apparently independent of the rhythmic contraction, can be observed in the weaker motion of extreme youth and age. Whether this occurs in the period of vigorous rapid motion could not be determined, but there would seem to be no reason why it should. It would seem to be a nervous action connected with weakness. The motion of the spermatozoid as a whole is comparatively slow and sluggish.

The movement of the living spermatozoids of *Ginkgo* was observed by Hirase, but only in a few instances and was not carefully described. Those of *Cycas* have as yet not been observed in a living condition. In September, 1898, Fujii (39) made a somewhat detailed study of the living spermatozoids of *Ginkgo*, and as his paper is published in Japanese and is thus inaccessible to many, an outline of his observations will not be out of place here.[1] As in the case of the writer's study of *Zamia* spermatozoids published in 1897, Fujii used a 10 per cent solution of cane sugar in studying the *Ginkgo* spermatozoids, and succeeded in keeping them living for several hours.

At 11.37 a. m. a spermatozoid escaped from a pollen tube and moved slowly, but with definite rate, for thirty minutes; afterwards it stopped and only moved its body at a definite place. At 1.05 o'clock p. m. only a ciliary movement was observed; at 1.30 p. m. it stopped all motion, as if dead, but soon afterwards it regained its ciliary movement, and finally at 2.05 p. m. it ceased all motion.

The second spermatozoid studied appeared from the pollen tube at 4.20 p. m. and moved in and out of the field of the microscope for one hour and twenty-five minutes. Afterwards, by a careless mistake, the sugar solution dried up and it stopped all motion. Besides these two spermatozoids I observed four others, but they lived only a short time. One spermatozoid observed by Mr. Yobe lived for three hours.

Fujii described the motion of the spermatozoids in swimming as similar to that of infusoria.

It is a very interesting fact, as observed by Fujii, that the spermatozoids occasionally cease all motion, as if dead, and after remaining in this quiescent condition for a time begin motion again. I have observed this many times in *Zamia*. Frequently the sperms swim very actively for a time and then cease motion, as if desiring rest, and later begin motion again. It would hardly seem probable that the sperm could absorb nourishment from the surrounding media and gain energy in this way for further motion, but this may be the case.

Later, in 1899, in a second paper, Fujii (40) described further observations on living spermatozoids and the methods by which they get out of the pollen tubes in sugar solutions. An extract from his description follows:

The spermatozoid in the mother cell moves its body gently and turns over in many directions by the ciliary movement, assuming various shapes, slightly changed by the simultaneous pressure of the mother cell and the two spermatozoids. At the same time, and owing to the same pressure, the nuclei also changed their form. The

[1] Fujii's papers were kindly translated for the writer by Dr. H. Ikeda.

spermatozoids being soft and very elastic, recover their shape after the pressure is past. The spermatozoids are next shown in figure 3 in the process of getting out of the mother cell, after which they swim freely in the pollen tube, from which they generally escape later. In general, there are two modes by which they get out of the pollen tube, viz, (a) gradually, (b) suddenly, and this difference depends chiefly on the density of the surrounding fluid. (a) Mode of gradually escaping: Figure 4 shows an example of a spermatozoid gradually getting out of the tube, observed in Tokyo. At 4.15 p. m. (September 17) the head of the spermatozoid made its appearance a little out of the pollen tube and after five minutes it entirely got out of the tube and recovered its normal shape. Then it swam with a definite rate until 5.45 p. m. From this observation it can be seen how soft and elastic the body is. This is very similar to the escape of the swarm spores of *Œgodonium* or the spermatozoids from its old cell wall, because also in this case the shape of the swarm spore or of the spermatozoid, before and after getting out of the old cell wall, are quite different, like that of the spermatozoid of *Ginkgo*. They also have an oval shape with numerous cilia. (b) Mode of suddenly escaping: In this case the membrane of the mother cell containing the two spermatozoids (also with Hirase's two cytoplasmic cylinders?) is suddenly thrust out of the pollen tube, as shown in figure 5 xxx. At first the shape of the spermatozoid is very irregular because of the surrounding pressure produced by sudden protrusion, but gradually it separates from the mother cell and takes the form shown in *b* and *b'* and ceases its motion for a short time. Finally it recovers its complete shape, like the fruit of an eggplant, and gently begins to swim with a definite rate. This fact also shows how soft and elastic it is. In a strict sense, however, it does not always recover its former shape. For instance, when it was subjected to great pressure at the time of escaping its shape becomes like that of a snail, and in such a case after getting out it will be killed by the deformation. But even in the case of suddenly escaping, if the pressure and change in shape is not too great, it will swim freely soon after escaping.

In the case of *Zamia* the writer has given considerable attention to the method of escape of the spermatozoids and has observed the two methods described by Fujii. The first method of the gradual creeping out of the spermatozoid has been frequently observed in pollen tubes placed in sugar solution, but in almost all cases the tubes could be observed to have been broken previously, thus allowing the elastic spermatozoid to stretch out and creep through without actually penetrating the membrane itself. In very numerous instances the extreme difficulty which the unwieldy spermatozoid has in overcoming slight obstruction has been observed; as, for instance, the difficulty of breaking the plasma membrane of the pollen tube described above (p. 53). In *Zamia*, where the cellulose wall of the pollen tube is quite thick, the writer is inclined to believe that it would be impossible for them to penetrate the wall in this manner. The second mode of escape by the sudden rupture of the pollen tubes is very commonly observed. At the time of maturity the tubes seem to be under great tension and a slight touch serves to cause them to burst and discharge their contents along with the spermatozoids as described in the writer's second preliminary paper (123, p. 18). This method of escape in *Zamia* would seem unquestionably to be the one normally occurring in the process of fecundation, as described hereafter.

Which end of the spermatozoid is to be considered the anterior end and which the posterior is not an easy question to determine in *Zamia*, if analogy with other forms is disregarded. In the pollen tube and in sugar solutions they move both backward and forward, and in their rolling, tumbling motion it is hard to recognize any system. However, there are several factors which enable us to determine that the apex of the spiral must be considered as the head end: (1) The two spermatozoids as developed are attached by the side opposite the apex of the spiral, and in their separation the cilia movement always exercises a very perceptible pulling force outward toward the apex of the spiral, which gradually results in the separation of the spermatozoids (figs. 47a to 47d). (2) In general, the selective end of the spermatozoid in free motion is the spiral end. (3) In slowly creeping out of broken pollen tubes, as described above, the spiral end usually precedes, but this is not always the case. (4) As observed over the neck cells of the archegonia, apparently in the process of attempting to enter, the spiral end has always been down toward the very small opening. (5) In entering the cytoplasm of the egg cell the apex of the spiral is always in the lead, as shown by very numerous instances (fig. 56).

The last two factors are of the greatest importance, and clearly show that the spiral end must be considered the anterior end. This, of course, is what would be expected from a comparison with the spermatozoids of ferns and mosses, but it is an interesting distinction from the animal spermatozoan where the motile organ forms the tail or posterior end.

In his preliminary paper announcing the discovery of spermatozoids in *Ginkgo*, Hirase (61) described the presence of a prolongation of the posterior end of the spermatozoid into a tail, an organ not present in other plant spermatozoids, so far as known. In the writer's preliminary paper in 1897, on the spermatozoid of *Zamia* (123, p. 20), it was stated that "there is no free tail in *Zamia*, as is said by Hirase to occur in *Ginkgo*." In his complete monograph in 1898, Hirase (62, p. 123) again describes and figures the spermatozoid with a sharp-pointed tail 28μ long. He says that since one never discovers the tail in the hemispherical spermatozoids at rest in the pollen tube, it seems rational to conclude that they are formed almost at the moment of the escape from the tube end at the expense of a certain portion of the cytoplasm. Ikeno (70, p. 579) was not able to study the living spermatozoids of *Cycas*, but concluded from a study of fixed material that the spermatozoids of *Cycas* corresponded to those of *Ginkgo* in the presence of a tail. He wrote, "auch ist ein spitziger Schwanz vorhanden, welcher weiter nichts ist als die Verlangerung des hinteren Endes des cytoplasmatischen Mantels."

The writer's conclusion in regard to the absence of a tail in the spermatozoids of *Zamia* were based on a study of hundreds of sper-

matozoids while swimming free in sugar solution, as well as on a study of prepared material, while apparently only two living spermatozoids had been observed of *Ginkgo* and none in the case of *Cycas*. This constituted one of the fundamental points of difference between the writer's conclusions and those of Hirase and Ikeno, which would seem to indicate a closer relationship of *Cycas* to *Ginkgo* than to *Zamia*. Fortunately this difference has been explained by Fujii (39), who undertook a study of the living spermatozoids of *Ginkgo* particularly to settle this point of difference. Fujii, as described above, studied a number of living spermatozoids, and had an excellent opportunity to settle this point of difference. He concludes as follows:

> Hence, with my observations, I shall infer that the spermatozoid of *Ginkgo* has no tail. Here I will only point out that, as everyone knows, Mr. Hirase did not draw a false figure different from what he observed, and I remember that his figure is the same as one which I saw in his preparation (which was afterwards unfortunately damaged during a journey). Therefore, I think that (1) the spermatozoid in Hirase's preparation was an abnormal one; (2) when the spermatozoid was getting out of the pollen tube possibly a broken part of another cell adhered to it, and he looked upon this as a tail; (3) he may have taken a part of the body of the spermatozoid broken by pressure with the cover glass to be a tail.

In a later paper on the same subject, Fujii (40) states that deformed spermatozoids having appendages similar to that described by Hirase are quite frequently observed, being caused by pressure in escaping from the pollen tube, etc. This can often be observed in the spermatozoids in the archegonia at the time of fecundation or later. He says:

> In this case we can observe the various shapes of deformed spermatozoids, and some of them seem to have a tail or nipple or a small lump at one end of the body; * * * hence, it is not a matter of surprise if Mr. Ikeno sketched a figure of a spermatozoid at the opening of the ovisac as having a tail-like appendage, and last October I affirmed, with the preparation made by Mr. Ikeno, that a part of the mantle of this spermatozoid changed and looked something like a tail.

Since the publication of his preliminary statement that no such tail was found in the spermatozoid of *Zamia* (123), the writer has carefully reinvestigated this question, using again both living and prepared material. All the evidence which has been accumulated strengthens his former conclusion, and he is certain that no tail is present in the spermatozoid of *Zamia* under normal conditions. As explained above, however, the body of the spermatozoid is highly elastic and has a more or less pronounced amœboid movement when swimming in a confined location like the interior of the pollen tube. It is easy to see that the instantaneous killing of the spermatozoid, when a portion of the body was extended, from pressure or otherwise, might result in obtaining preparations showing tail-like protrusions. Such deformities are rarely met with, however, in *Zamia*, being evidently of much rarer occurrence than in *Ginkgo*. Ikeno's evidence supporting the occurrence of a tail in *Cycas* is evidently based on a few instances of the

appearance of some such an organ on the posterior end of the spermatozoid after it has entered the archegonium. In passing through the very narrow entrance canal between the neck cells, the spermatozoids have use for all possible elasticity, as they must from necessity stretch out into a very long, slender form in making the passage. The act of passage has not been observed in *Zamia*, but the spermatozoids have been observed in many cases just after the passage. In one or two instances material has been found adhering to them which might have been interpreted as a tail had no other spermatozoids been observed. The adhering substances in these cases seemed to be from the granular mucilaginous matter in which the spermatozoids swim. Usually the spermatozoids found in the archegonia have assumed their normal form and show no suggestion of the extreme pressure they must have endured in the process of entering.

In conjunction with Mr. E. A. Bessey[1] the writer has made some interesting observations on the living pollen tubes of *Ginkgo* mounted in 10 and 5 per cent sugar solutions. The whole pollen tube apparatus is clearer and much more favorable for observation in the unstained condition than that of *Zamia*. In very many tubes the main features of the central cell could be plainly observed. The nucleus *corps sphériques* and the blepharoplasts were plainly visible when tubes were cut off and placed in sugar solutions without injury. The nucleolus could almost invariably be observed to contain at least one large vacuole, and very frequently a number of smaller ones could be observed. In one instance a nucleus was observed in a prophase of division when the chromatin had collected in a skein. This was an absolutely fresh tube and could not be mistaken, being as plain as in the fixed and stained material. The nucleoplasm outside of the skein, which was composed of large granules and presented a dense appearance, was clear and finely granular, the only difference between the skein and other nucleoplasm seeming to be the larger size of the granules in the skein and their greater density. In quite a number of cases the blepharoplast could be plainly distinguished, and in one instance shown to the writer by Mr. Bessey the contents could be seen to be vacuolated or reticular almost as plainly as in the fixed and stained material. There can thus be no doubt that the main features brought out by fixing and staining in *Ginkgo* and *Zamia* are perfectly normal and not artifacts. The study of the living material of *Ginkgo*, which should be carried much farther, promises much valuable information, particularly in confirming the results obtained by fixing and staining.

Personally the writer has made no attempt to secure the living and

[1]These observations on the living material of *Ginkgo* were made on material collected by Mr. E. A. Bessey, one of the writer's colleagues. He also made many interesting observations, a short account of which will be found in Science, February, 1901.

moving spermatozoids of *Ginkgo*, but he has fortunately been able to observe and study some of those obtained by Mr. Bessey, and can confirm the main features of the movement as described by him. The tail-like appendage which was described by Hirase certainly does not exist in the normal spermatozoids, and in the specimens observed by Hirase was doubtless due to compression in escaping from the pollen tube or some similar cause, as suggested by Fujii. The shape of the spermatozoids which have been observed by Mr. Bessey and the writer corresponds well with those figured by Hirase and Fujii. The movements of the spermatozoids of *Ginkgo* are almost exactly the same as those observed by the writer in *Zamia*. The amœboid movement of the apex of the spiral is very noticeable in *Ginkgo* also, and the rythmic motion of the cilia, similar to that occurring in *Zamia*, has been observed by Mr. Bessey.

A very interesting observation, first made by Mr. Bessey and also studied by the writer, is the rythmic vibration of a portion of the membrane at the base of the spermatozoid corresponding with the vibration of the cilia. The spot is apparently just over the "*corps sphérique*," and may have some relation to that body.

PROCESS OF FECUNDATION.

While the spermatozoids have been maturing, the proximal ends of the pollen tubes, as described above, have been growing down through the tissue of the nucellus into the archegonial chamber above the archegonia. When all of the organs are developed ready for fecundation the pollen tubes hang down so that the ends almost or quite touch the neck cells of the archegonia, which protude into the same cavity. It is interesting to note that the pollen tubes when they enter the archegonial chamber (endosperm or prothallial cavity), which seems to be filled simply with moist air, do not grow at random, but bend slightly outward and grow directly toward the neck cells of the archegonia. Frequently several were observed to grow toward the same archegonium. These observations can be made on living material by carefully cutting into the archegonial chamber at one side, without injuring the tubes, and observing them with a hand lens. The end of the tube is occupied by the spermatozoids and the vegetative cells of the male prothallus. It is probable that the spermatozoids normally begin swimming in the tubes before the latter burst, as they have several times been observed swimming in the unbroken tubes. The end of the pollen tube is wider than the upper portion and is evidently under considerable tension. The protruding tip formed by the old pollen grain (figs. 47 and 51) is plainly visible with a hand lens, and is evidently the point which first comes in contact with the neck cells of the archegonium. The neck cells are also distended and turgid and are evidently easily broken. If in this distended condition the end of

the pollen tube be touched very lightly with the flat side of a scalpel it bursts, and the spermatozoids, together with a drop of the watery contents of the pollen tube, are quickly forced out and the pollen tube immediately shrivels up into a shapeless mass. This the writer thinks is what happens in the normal course of fecundation. The pollen tube evidently grows down until the end is forced against the neck cells, when the tube bursts, discharging the mature spermatozoids and the watery contents of the tube, which supplies a drop of fluid in which the spermatozoids can swim. Some doubt exists as to whether the pollen tube supplies all of the fluid in the archegonial chamber at the time of fecundation or whether some of it is extruded by the egg cell.

Hirase (62, p. 122) described the occurrence of a sap filling the archegonial chamber at the time of fecundation, and considered that it was very probably a product of the female organ. No evidence is given, however, to show that this is the case. Ikeno (70, p. 583) also thinks that the fluid is largely a product of the female organ. He says:

> I have often met with cases in *Cycas* in which fluid was already present, in spite of the fact that all pollen tubes were yet entirely intact; so that we are led to the conclusion that at least a part of this fluid—probably the larger part—proceeds from the female organ.[1]

If Ikeno was not mistaken in his observation, he is certainly correct in claiming that the female organ furnishes part of the fluid. In *Zamia*, however, the writer has frequently observed that the numerous pollen tubes are in various stages of development. One tube may have the spermatozoids swimming about in it, while in an adjoining tube the central cell has not yet completed its division. When a tube has burst and dicharged its spermatozoids it shrivels into an unrecognizable, small, and almost indiscernible mass. Tubes in an advanced stage may burst and leave a liquid in the archegonial chamber, while other tubes remain entire. In *Zamia* a portion of the fluid is certainly furnished by the pollen tube. Whether any of it is furnished by the female apparatus the writer has been unable to positively determine. It may be so, but the neck cells remain turgid and fresh up to the very time of fecundation, and no indication of the beginning of the exudation of a fluid has been observed, though it would seem that an abundant opportunity has been furnished for observing such an exudation if it occurs.

The writer has several times observed the spermatozoids after they were discharged over the archegonia, but studying them in this position is unsatisfactory and difficult. They have been observed to swim

[1] Ich habe bei *Cycas* oft Fälle angetroffen, in denen eine Menge Saft schon in der Endospermhöhle vorhanden war, wenn auch alle Pollenschläuche noch ganz intact waren, so dass wir zu der Annahme geführt werden, dass wenigstens ein Theil dieses Saftes—wahrscheinlich der grösste Theil—aus dem weiblichen Organe herstammt.

to the neck cells and stop over these and continue to gradually revolve around and around, apparently in the process of crowding into the egg cell. In fecundation the entire spermatozoid unchanged swims into the egg cell, passing between the ruptured neck cells. The entrance tube is very narrow compared with the size of the spermatozoid, and the latter must be greatly stretched out in accomplishing the passage. It is certain that they pass through into the egg cell entire, as they have in many instances been found in the egg cell having their normal shape. Several spermatozoids commonly enter each egg cell, two and three having been found in very many instances. Only one of these takes part in fecundation, and the others may be found presenting a perfectly normal appearance or in some stage of disintegration. Those not concerned in fecundation may usually be found in the upper part of the egg cell between the wall and the cytoplasm, which is slightly contracted away from the wall in the majority of the writer's preparations. In some instances they seem to have crowded against the cytoplasm of the egg and caused a noticeable indentation (figs. 55 and 70). Occasionally one of the spermatozoids not concerned in fecundation pushes for a short distance into the contents of the egg cell, but such spermatozoids do not mingle with the protoplasm of the egg cell, as they are always found in such cases to form distinct bodies, which stain very differently and remain intact until long after fecundation has taken place. The spermatozoid which reaches the egg cell first would seem to be the one which causes fecundation. That one which is utilized in fecundation swims into the cytoplasm of the egg cell for a short distance, where it comes to rest and undergoes change. The nucleus slips out of its cytoplasmic sheath and passes on alone from this point to the egg nucleus, with which it unites. The spiral ciliferous band, which forms such an interesting part of the spermatozoid, remains at the apex of the egg cell in the place where the nucleus left it. In very numerous instances just after fecundation it has been discovered in this position, and there can be no doubt that this process is the one normally occurring. It shows very plainly and presents nearly the original form of the spermatozoid, but is always stretched out much more than in the normal spermatozoid. The band lies free in the cytoplasm of the egg cell, and the sections of the spiral, with the numerous cilia radiating from them, are frequently very distinct and can be easily photographed (fig. 69).

The method of the escape of the nucleus from the body of the spermatozoid can only be conjectured. It would seem, however, that the rapid boring of the apical or spiral end into the egg cell may cause too great a pressure on the large body of the spermatozoid, resulting in its bursting and freeing the nucleus, while the cilia motion continues probably some time longer, carrying the band farther along and freeing the nucleus from any hindrance by it. The apex or spiral end of the

spermatozoid invariably enters the egg cell first, and in all of the cases observed where the nucleus has just escaped from the spermatozoid it has been found a short distance behind the spiral of the spermatozoid, as if it had been forced out and left behind (fig. 54). The function of the cytoplasm of the spermatozoid is still in considerable doubt, but that it fuses with the cytoplasm of the egg cell is certain. Shortly after the nucleus has broken out of the spermatozoid cell the thin layer of dense cytoplasm which surrounded it can be seen in a broken, fragmentary form, still somewhat connected with the spiral band (fig. 57). The cytoplasm of the spermatozoid in this stage is very different from that of the egg cell, being more densely granular and staining more deeply, so that it is easily distinguished. Later only a rather coarse granular substance is found inside of the spiral coil of the ciliferous band, and it would seem that this is the cytoplasmic matter from the spermatozoid which has mingled with that of the egg cell. It should be mentioned that the plasma membrane surrounding the spermatozoid has entirely disappeared, no trace of it being visible. It would seem to have fused with some substance of the egg cell or to have been absorbed in some way.

No case of polyspermy has been observed in the specimens examined. In no instance has more than one empty spiral been found in the same egg cell. Where an empty spiral was found it could be predicted that the egg nucleus would be found to have been fecundated; and, vice versa, when a fecundated egg nucleus was found it could be predicted that an empty spiral ciliferous band would be found at the apex of the cell. No exception to this rule was observed in the very large number of specimens examined.

The male nucleus, when it has escaped from the spermatozoid and is observed lying in the cytoplasm at the apex of the egg cell, is of loose, open structure, seeming to have but little kinoplasmic and chromatin matter. The passage to the nucleus is evidently a rapid one, as few stages have been found between the above and the completion of fecundation. In some instances the path over which the nucleus traveled in reaching the egg cell is discernible by the arrangement of the granules in the cytoplasm, showing the direction of the passage (figs. 55 and 56).

The egg nucleus previous to fecundation is elliptical and is located slightly below the center of the enormous egg cell, which is about 3 mm. long by 1.5 mm. wide. The egg nucleus is of enormous size, comparatively, being plainly visible to the unaided eye. It is composed of an open, coarse reticulum (fig. 54). So far as the writer has observed, there is no depression or "empfängnisshöhle" in the upper part of the nucleus where the sperm nucleus enters, as was found by Ikeno in *Cycas* (70, p. 585). No special attention has been given to this matter, however, and further observation may show it to be present. The male nucleus in entering the egg nucleus gradually pushes

into it, as observed by Ikeno in *Cycas*, and finally becomes entirely surrounded by it. Meanwhile it has changed its structure and become densely granular, differing markedly from the egg nucleus in this particular. Considerable search has been made for indications of extrusions of matter from the sperm or egg nucleus at the time of fecundation, such as has been described by Ikeno in *Cycas* (70, p. 587). No indications of such extrusions have been found, however. After fecundation is apparently completed, the male nucleus appears as a small, nearly round body in the upper portion of the egg nucleus into which it has penetrated. The further changes in the male and female nuclei before they undergo division have not been followed.

The isolated ciliferous band lying free in the protoplasm at the apex of the egg cell evidently retains its identity for a considerable time. It has been observed in several instances after the formation of many free nuclei by the repeated divisions of the oosphere. Frequently the spindles of some of these free nuclei in division have been observed between its spirals. The band ultimately disappears, its substances probably being consumed by the forming embryo. The primary function of the ciliferous band thus certainly ends with the transporting of the male germ cell from the pollen tube to the egg cell, as was first shown by the writer in October, 1897 (124). The same process of fecundation was later described by Ikens as occurring in *Cycas* (69). The exceptional size of the spermatozoids and egg cell in *Zamia* permits these features to be seen very plainly. While in the majority of plants in which the entrance of the spermatozoids has been studied, they are so small that thus far the fate of the cilia and cytoplasm, which are not generally supposed to be concerned in fecundation, has not been determined with very great certainty. In *Fucus* Strasburger (111, p. 363) has concluded, judging mainly from comparative size, that shortly after the entrance of the spermatozoid its cytoplasm unites with that of the egg cell and only the nucleus continues its passage and unites with the egg nucleus. Shaw's studies of the fertilization of *Onoclea* (103) indicate that in fecundation the cytoplasm and cilia-bearing band remain in the cytoplasm of the egg cell, but this was unfortunately not definitely determined. It is interesting to note further in this connection that the spermatozoid nucleus in *Onoclea* unites with the egg nucleus without any change of form. Thom's study of the process of fertilization in *Aspidium* and *Adiantum* (115) is also very interesting in this connection. It would seem from Thom's investigations that commonly the entire spermatozoid enters the egg nucleus, although this is not made plain, as it is stated that "the cytoplasmic forward end contains, or is partially derived from, the so-called blepharoplast, and bears numerous long cilia. This part either becomes disconnected entirely before the spermatozoid reaches the egg, or, becoming functionless, is turned backward and dragged passively along into the cytoplasm of the egg."

This would seem to confirm the writer's statement that the blepharoplast is an organ developed primarily for the transportation of the male nucleus, but the discrepancy between the question of fusion of cell parts is very noticeable. In *Zamia* and *Cycas* the entire spermatozoid invariably enters the egg cell and the cytoplasm and ciliferous band (blepharoplast) fuse with the cytoplasm of the egg, while the nucleus journeys on to the egg nucleus, with which it fuses. A number of investigators, the writer has noticed, seem to conclude that because the nucleus of the spermatozoid travels on alone and fuses with the egg nucleus the nucleus alone is concerned in fecundation. It may be that the nucleus is the sole bearer of hereditary tendencies and that this is the important part of fecundation. The fact remains, however, that in *Zamia* and *Cycas* and those cases of fecundation that are best known there is a fusion of cells, nucleus with nucleus and cytoplasm with cytoplasm, as would be naturally expected. We could hardly expect the entire spermatozoid nucleus and cytoplasm to fuse with the egg nucleus. It would seem as though the cytoplasmic envelope figured by Thom, as left by the spermatozoid in the cytoplasm of the egg cell after the nucleus has escaped, must contain the ciliferous band, if, indeed, it is not made up almost entirely of the band. From analogy with *Zamia* and *Cycas* this would immediately be supposed to be the ciliferous band of the spermatozoid, the nucleus having united with the egg nucleus and the cytoplasm with the egg cytoplasm.

In the Gymnosperms, according to Blackman, Murrill, and others, it is an entire cell that takes part in the fecundation, but no blepharoplast is here present. Dixon (27) was the first to observe in *Pinus sylvestris* that all four nuclei from the pollen tube—the two generative nuclei, the pollen tube nucleus, and the nucleus of the stalk cell—passed over into the egg cell in fecundation. Blackman (16) confirmed this conclusion in his study of *Pinus sylvestris*, and further stated that "it can not be doubted that cytoplasm also passes over into the oosphere, for each generative nucleus in the pollen tube is clearly surrounded by its own layer of cytoplasm, as can be observed in the stage when the tube is clearly in contact with the oosphere." Murrill, in his study of *Tsuga canadensis* (91), claims that the contents of the pollen tube "cast into the egg consists of two sperm cells, the stalk cell, the vegetative nucleus, and some protoplasm and starch from the tube cavity." The sperm cells are described as having dense cytoplasmic contents and large nucleus. The process of fecundation described by Murrill compares exactly with what the writer had previously described in *Zamia* (124) and what Ikeno found in *Cycas* (70). Murrill says:

> It is through the first sperm nucleus that fertilization is accomplished. A short time after its entrance into the egg it slips from its cell and moves with accelerated velocity toward the egg nucleus, the latter remaining stationary and inactive. * * *

In the case of the spermatophytes also some recent investigators are claiming that the male germs which pass over into the egg cell are true cells and not simply nuclei. The discovery of the spermatozoids of *Ginkgo*, *Cycas*, and *Zamia*, and the demonstration of the action of these enormous spermatozoids in fecundation, has had much to do in clearing up our ideas of fecundation, as it was in *Zamia* and *Cycas*, where for the first time it was positively shown in plants that an entire male cell entered the egg and the cytoplasm fused with the cytoplasm of the egg cell, while the nucleus traveled on and fused with the egg nucleus.

At the time of fecundation in *Zamia*, before the formation of the primary spindle has begun, so far as can be told, a very peculiar condition of the cytoplasm is observed throughout the enormous egg cell. The entire kinoplasm of the cell seems to collect in little comet-like figures here and there throughout the cell, presenting a most remarkable appearance (fig. 69). This condition is observed in sections stained by both the Flemming and the Haidenhein methods. Sections showing this polarized condition of the kinoplasm were exhibited at the meeting of the British Association for the Advancement of Science, at Toronto, Canada, in 1897, and excited interesting comments. The kinoplasmic rays here seem to run together at one point, but there is no differentiated body occupying this point upon which they are focused. There seems to be no regular direction in which the rays extend. They are here and there and all over, throughout the egg cell, without any regularity. They can not come from the broken-down ciliferous membrane, as it still remains perfectly intact at the apex of the egg cell. They can not be fragments of the spindle resulting from the division of the canal cell, for they are never observed previous to fecundation. Chamberlain (21, p. 277, figs. 8 and 32) illustrates cytoplasmic comet-like figures in the egg cell of *Pinus laricio* much like those which the writer has found in *Zamia*, but he thinks them to be broken-up portions of the spindle formed in the cutting off of the ventral canal cell. This the writer thinks is certainly not their origin in *Zamia*. Their function also remains in doubt, but it would seem probable that they have some important function in the formation of the first segmentation spindle.

DIVISION OF THE FECUNDATED EGG CELL.

In establishing the complete history and nature of the blepharoplast it is of special interest to determine whether it has any important function in the division of the fecundated egg cell. If it is a centrosome, as claimed by some writers, does it function as a centrosome in the segmentation of the egg? It seems to have been established that in many animals the spermatozoid brings in the centrosome which forms the amphiaster for the first division, but this has not yet been proved in the case of any plant. That the blepharoplast or the cilia-

forming organ enters the egg and remains in the cytoplasm in *Zamia* and *Cycas* is certain and not open to any question.

The writer has endeavored by diligent search to observe the formation of the first segmentation spindle, but thus far has been unable to succeed. However, he has been able to observe the spindle in the second division which leads to the formation of four daughter nuclei. Here the spindle is of normal form, but rather drawn out at the poles. No differentiated body can be observed at the poles of the spindle which could be considered a centrosome. In the later divisions also very many dividing nuclei in various stages have been carefully studied, but without success. In no case of dividing nuclei in the early cleavage divisions before the seed matures has any centrosome been observed. This statement corresponds entirely with the writer's earlier conclusions reached in 1897 (124). In the case of *Ginkgo* and *Cycas* also, according to the researches of Hirase and Ikeno, no centrosomes or centrospheres could be found in the early divisions of the egg nucleus.

Aside from this very conclusive evidence that the blepharoplast brought in by the spermatozoid does not form a centrosome which takes part in the formation of the first segmentation spindle, the conclusion is indubitably established furthermore by the fact that the ciliferous band remains intact at the apex of the egg cell for some time following the division of the egg nucleus. It has been observed unbroken after at least five or six divisions when the daughter nuclei had become spread here and there in the egg cell. In several instances nuclei resulting from the division of the egg nucleus have been found occupying a position between the spirals of the ciliferous band. This was very puzzling when first discovered before the development was understood.

The division of the egg nucleus results in a decided reduction in the size of the nuclei until they are reduced from the tremendous size of the egg nucleus, which is visible to the unaided eye, to rather small nuclei not above an ordinary size. The first two or three divisions take place while the nuclei remain grouped together in the center of the egg cell in the position of the original egg nucleus; after this the nuclei become gradually scattered throughout the egg cell and finally, in the first stage of the organization of the embryo, form a layer of cells around the periphery of the egg cell. The history of the formation and development of the embryo, however, has no place in the present memoir. The writer will discuss this matter at some future time in another place.

IS THE BLEPHAROPLAST A CENTROSOME?

The feature of most interest in this investigation is the question regarding the true nature of the blepharoplasts or cilia-forming organs of the spermatids. When these organs were first observed in *Ginkgo* by Hirase in 1894 (57) he referred to them as attractive spheres.

would naturally be done by anyone unfamiliar with their complete history. The next reference to them in literature occurs in the writer's first preliminary paper published in June, 1897 (122), in which their centrosome nature is questioned. In the writer's third preliminary paper, which appeared in October, 1897 (124), it was shown for the first time that the bodies in question originate *de novo* in the cytoplasm of the central cell, apparently having no important functions in the formation of the spindle, when this cell divides to form the spermatids, and after it has served its function in forming the cilia of the spermatozoid it disintegrates at the apex of the egg cell, apparently having no further function. For these reasons it was concluded that the organs were not centrosomes proper, and they were termed *blepharoplasts*, because of their special function as cilia-formers. This immediately led to controversy, and the question is still unsettled. Early in 1898 Ikeno (69, p. 17) stated unreservedly that the centrosome-like body in Cycads and *Ginkgo* is a true centrosome. He said:

> If we apply this conclusion of Hermann to our case, then it is quite clear that the body in question, which corresponds to the middle piece in serving as the cilia-bearing thread, is not only similar exteriorly to a centrosome, but is a true centrosome, and that the cilia-bearing thread is to be regarded as an enormously enlarged centrosome.[1]

This opinion is further emphasized by Ikeno in his complete monograph on the fecundation of *Cycas revoluta* (70). Hirase, in his study of *Ginkgo* and the attractive spheres formed in the spermatogenous cells, also concludes that they are to be considered as centrosomes, though, as shown by his figure 18 (61, pl. 8), they remain distinct from the spindle, the radiations around the sphere not connecting with the radiations around the pole of the spindle in the center of which a centrosome should be located, if present. Hirase says, furthermore:

> The attractive spheres which we have just described are different from those made known by many scientists heretofore. In the first place, they differ in that they are always at a certain distance from the poles of the spindle, and in the second place that in the course of karyokinesis they do not divide into two daughter spheres.[2]

Guignard also takes the same ground, considering the writer's researches on *Zamia* as proof of the existence of centrosomes in seed plants. He says:

> Even though all earlier observations upon the presence of attractive spheres and centrosomes in different Cormophytes may be regarded as inexact, one can not doubt that the bodies recently described and figured by Webber in the pollen cells of *Zamia* * * * are centrosomes (48, p. 161).

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[1] Ueberträgt man diese Hermannische Folgerung auf unseren Fall, so ist es ohne Weiteres klar, dass der in Rede stehende Körper, welcher sich zum Mittelstück entsprechenden cilientragenden Faden ausdehnt, nicht nur äusserlich einem Centrosom ähnlich, sondern ein wahres Centrosom ist, und dass der cilientragende Faden als ein enorm herangewachsenes Centrosom zu deuten ist.

[2] ...attractive que nous venons de décrire sont différentes de celles signalées ...ieurs savants, en premier lieu, en ce qu'elles sont toujours ...les du fuseau, et en second lieu en ce qu'au cours de la ...se en deux spheres-filles.

Balajeff also, in his recent researches on *Marsilia*, concludes that the blepharoplast must, from its position and relation to the achromatic spindle, be considered a centrosome. He says:

In this manner the stainable corpuscles possess all the peculiarities characteristic of the centrosomes, not only as a result of their position at the poles of the spindle, but also through their relation to the achromatic threads.[1]

Practically the same conclusion in regard to the homologies of the blepharoplast is reached also by Chamberlain, who states it thus:

It seems probable that a thorough investigation of karyokinesis and the formation of cilia in the lower plants may support the theory that the blepharoplast is a centrosome (20, p. 434).

E. B. Wilson also regards the blepharoplast as the homologue of a centrosome or centrosphere. He says:

The later studies of Shaw (102) and Belajeff (14, p. 199) on the blepharoplasts in *Onoclea* and *Marsilia* leave no doubt that these bodies are to be identified with centrosomes (130, p. 175).

The writer in his studies has not been blind to the fact that the bodies in question resembled the centrosomes or centrospheres which have been described by some authors, both in external appearance and function. Our idea of the centrosome as a permanent *sui generis* organ of the cell, having as its prime function the governing and controlling of cell division, has become so modified in the last few years that it is hardly possible to define what constitutes a centrosome. It seemed to the writer that it was high time that organs resembling a centrosome which could be proven to have very definite and distinct functions from the centrosome as ordinarily understood should be given distinct names, whether or not they can ultimately be traced back and found to be homologous organs. We do not call the supporting tendril of the Virginia creeper a leaf, nor the leaf a tendril, yet they are clearly homologous organs. It was from this standpoint that the writer was willing to brave the odium of introducing another new term to our already crowded vocabulary. The blepharoplast, it is true, may ultimately be proved to be the homologue of a centrosome, and the writer forcibly called attention to this possibility at the Ithaca meeting of the American Society for Plant Morphology and Physiology, held in December, 1898. Even if this were true, however, which the writer is still inclined to doubt, it would nevertheless be necessary to have a distinguishing term, as the organ has now assumed a specialized function different from the original. The writer's view that the blepharoplast is probably a distinct organ from the centrosome has received the support of Shaw (102), Mottier (89), Strasburger

[1] Auf diese Weise besitzen die färbbaren Körperchen nicht nur in Folge ihrer Lage an den Polen der Kernspindel, sondern auch durch ihre Beziehungen zu den achromatischen Fäden alle Eigenthümlichkeiten, welche den Centrosomen charakteristisch sind, sie müssen daher als solche betrachtet werden (14, p. 202).

(112, p. 185 *et seq.*), and Studnicka (113), whose conclusions will be mentioned in some detail later on. Before stating these, however, it will be well to compare the blepharoplasts of the Cycads, *Ginkgo*, and ferns with some of the cases of typical centrosomes and centrospheres. An exhaustive comparison would extend this paper beyond its desired limits and would be of questionable value, because the centrosome question as a whole is in too great confusion to allow any final conclusion to be reached.

One of the most typical cases of centrosomes in plants is that described by Swingle in 1897 as occurring in *Stypocaulon* (114). Here a minute, deeply staining, dumb-bell-shaped body occurs at the pole of the spindle in karyokinesis, which at the close of division divides into two. Both of these remain in close connection with the nuclear membrane, but travel in opposite directions until they come to lie at opposite points on the equator of the nucleus. They are always surrounded by rays of kinoplasm, which become very abundant during spindle formation and division and are not surrounded by any differentiated sphere of any sort, as so commonly occurs in the centrosomes of animals. In spindle formation the centrosome appears to be of prime importance, a bundle of fibers starting from each centrosome and gradually extending into the nucleus until the spindle is completely formed. Swingle's studies were made with growing vegetative tips, which would indicate that the centrosome here is probably a permanent organ in all stages of growth. Centrosomes of almost exactly the form of those of *Stypocaulon* have been described by Strasburger (111) in *Fucus*. A very distinct deeply staining centrosome is described, which he believes to be a permanent organ of the cell, reproducing by division at the end of each nuclear division, thus forming two which control the next division. The observations of Farmer and Williams (37) are also of interest in this connection. They describe very marked centrospheres at the poles of the spindle of *Fucus*, in which an irregular number of granules, possibly representing centrosomes, can be observed. The number and general character of these granules is not uniform and the writers do not attach any significance to them. The centrospheres could not be traced through the resting cell, and are apparently originated *de novo* at each period of nuclear division. The relation of the centrospheres to spindle formation was not traced out in detail, but its connection with the mature spindle and later stages of division is unmistakable. In fecundation no visible centrosphere is brought into the egg by the spermatozoid, and Farmer and Williams find no support for the statement of Strasburger that an apparent connection can be traced between the position of the two centrospheres of the dividing egg and the limits of the portion of the oospore nucleus which belonged to the sperm. The connection of the centrosphere with cilia formation in the spermatozoids of *Fucus* has not been traced out,

but it is certain that the centrospheres occur here before and after fecundation in connection with spindle formation and karyokinesis, and are not simply cilia-forming organs of the spermatozoids, if indeed they can be in any way identified with such a function.

In *Dictyota*, also, Mottier (89 and 90) has demonstrated the occurrence of centrosomes similar to those described in *Stypocaulon* by Swingle, and in *Fucus* by Strasburger. They are small, deeply staining bodies, located in the center of a large aster, and are apparently permanent organs of the cell, reproducing by division during the reconstruction of the daughter nucleus. The centrosomes here also are intimately connected with spindle formation, as in the case of *Stypocaulon*, the fibers growing into the nucleus from each centrosome in forming the spindle. In the above three cases, *Stypocaulon*, *Fucus*, and *Dictyota*, there is a great uniformity of the centrosomes and their action and appearance, and they are by all means the best worked out, most definite, and positive cases of centrosomes known to occur in plants, though many other cases of centrosomes and centrospheres, etc., have been described. To these the blepharoplasts of *Zamia* have only a very indistinct resemblance in being located at the center of a group of radiations. In all essential features they are totally distinct.

In the division of the tetraspore of *Dasya* Davis (25) has described the occurrence of a body at the pole of the spindle which is supposed to be a centrosome or centrosphere which in one stage is broken up into a mass of granules, in this regard resembling the blepharoplasts of *Zamia*. In other ways and in function they are apparently very distinct organs from the blepharoplasts.

In the fungi several cases of well-authenticated centrosomes or centrospheres have been described, but all of them are very distinct from the blepharoplasts of *Zamia* and the Cycads. In the nuclear division in the ascus of *Erysiphe* Harper, in a brilliant contribution, has described the presence of a centrosphere which takes part in the formation of the plasma membrane of the spore. In the nucleus just previous to division, the centrosphere forms a flattened disk attached to the nuclear membrane. Later this disk becomes surrounded with numerous radiations. The division of the centrosphere has not been observed here, but stages slightly before the division, when the two daughter centrospheres are still near together, are figured by Harper (51, p. 251, figs. 4, 5, and 6). The centrosphere here would seem to be a permanent organ of the cell, increasing by division, but this is yet uncertain. Its connection with spindle formation is plainly evident, the fibers growing into the nucleus from it toward the chromosomes and finally forming the spindle. In spore formation in the ascus the centrosphere was found by Harper to have the novel function of forming the plasma membrane delimiting the spore in the ascus, a method of free cell formation which has been observed in no other place, so far as the writer

is informed. This is accompanied by a neck extending out from the nucleus at the point where the centrosphere is located, the centrosphere being extended away from the main body of the nucleus farther into the cytoplasm. When this neck or beak has reached its definitive length the kinoplasmic rays all bend downward and come to lie in a plane parallel to the nuclear wall and fuse together, forming a bell-shaped membrane surrounding the nucleus, with the centrosome forming its apex. By the growth of this membrane the nucleus is finally entirely surrounded, together with a portion of the cytoplasm of the original ascus, and the ascospore delimited. The same process of free cell formation in the delimiting of spores in the ascus has also been carefully described by Harper as occurring in *Lachnea* and is probably a common method of spore formation in asci. The extension of the beak from the nucleus which remains in connection with the centrosphere while the kinoplasmic rays from the latter fuse together and form the plasma membrane delimiting the ascospore, is similar to the beak from the nucleus of *Ginkgo* and *Cycas* which Hirase (62) and Ikeno (70) have found to remain in connection with the blepharoplast while it is extending in length and forming the cilia of the spermatozoid. This beak connection also recalls the beak of the nucleus which extends out to the *Mundstelle* on the plasma membrane of the cell in the formation of the cilia of the swarm spore of *Vaucheria* as described by Strasburger (112, p. 188). That there is an analogous relation between the nucleus and the centrosphere, blepharoplast, and *Mundstelle*, respectively, in the three cases can not be questioned.

In the Hepaticæ centrospheres have been described by Farmer (33) as occurring during spore formation. They form the center of a series of radiations, and do not become visible until the radiations are fairly well developed. The center of the system of radiations was not always occupied by a single granule or centrosome; often several distinct granules were visible, forming a microcentrum in the Heidenhain sense. The centrospheres disappear at the close of the division, and before each division are apparently originated *de novo* in the cytoplasm of the cell in close relation to the nuclei. The interesting feature in connection with the centrosomes here and the blepharoplasts in *Zamia* is that they are supposed to originate *de novo* in the cytoplasm of the cell. Studies of spermatogenous cells of the Hepaticæ would doubtless prove of special interest, as a genuine centrosome being present in the divisions during spore formation may also be expected to occur in these divisions as well.

The cases of centrosomes among higher plants, or spermatophytes, are all as yet open to some degree of doubt. Various authors have claimed to have found special granules at the poles of the spindle, and that this is the case can hardly be questioned. In the divisions leading to the formation of the pollen in *Nymphæa*, *Nuphar*, and *Limo-*

durum, Guignard (49) describes the occurrence of definite granules at the poles of the spindle which seem to be similar to centrosomes. He claims that the occurrence of multipolar spindles can not be taken as evidence of the nonoccurrence of centrosomes, as the multipolar spindle in which a definite granule may occur at each pole evidently later becomes bipolar by the various polar ends of the spindle swinging around together and uniting in such a manner as to form a normal bipolar spindle. Schaffner also claims to have found bodies which he calls centrosomes at the pole of the spindle in *Sagittaria* (99), and in root tips of *Allium cepa* (100). Fullmer also claims to have found centrosomes in the seedlings of *Pinus larico* and *P. sylvestris* (41). Even considering the claims of Guignard, Schaffner, and Fullmer for the presence of a centrosome in certain Spermatophytes, their occurrence is still a question of grave doubt. The very careful and complete researches of Osterhout (94), Mottier (87, 88, etc.), and many others, where no indication of a centrosome has been found, throw a doubt on the matter, and their presence must be confirmed by other investigators in those plants where they are said to occur before their normal and regular occurrence can be credited. They must be of such a nature that they can be demonstrated to occur in the same species of plant in the same stage of development by various investigators. If we are to recognize evanescent bodies as the homologues of centrosomes, our whole idea of the importance and nature of these organs must change.

Our conception of the centrosphere and centrosome is continually changing. The original idea of Boveri (17) that the centrosome is "a distinct, permanent cell organ, which, increases by division and supplies the dynamic centers for the succeeding cell formations,"[1] has been greatly modified by the extensive researches of recent years. It is no longer looked upon as a necessary cell organ reproducing itself by division, a number of instances being known where they are formed *de novo* in the cell. Various forms are also known, so numerous that there seems to be almost no correspondence between them; still there are certain morphological characteristics and certain functions which may be said to be common to all centrosomes. The centrosome or centrosphere, in its typical sense, as the writer understands it, is an organ of the cell, with the following attributes: (1) It is located in the center of an aster, at the pole of the spindle during division; (2) it has, as its special function the formation of the spindle and the control of the division; (3) it occurs usually, at least, in the division of sexual and embryonic cells.

In regard to the first of these propositions the writer is not aware

[1] "Ein der entstehenden Zelle in der Einzahl zukommendes distinktes dauerndes Zellenorgan, das, durch Zweitheilung sich vermehrend, die dynamischen Centren für die Entstehung der nächst zu bildenden Zellen liefert" (17, p. 60).

that any cell organ, which never forms the center of an aster at the pole of the spindle, has been considered by any investigator as the homologue of a centrosome. An exception, of course, must be made in the case of the blepharoplasts of *Zamia*, *Cycas*, and *Ginkgo*, which are under discussion, if we accept the statement that they are at the poles of the spindle. The blepharoplasts of *Marsilia*, according to Belajeff's investigations, are located at the pole of the spindle, but, judging from his figures, they are not located in the center of an aster.

In regard to the second proposition, it may be stated that in all of the well-worked-out cases in plants where centrosomes or centrospheres occur, as in *Sphacelaria*, *Dictyota*, *Fucus*, Hepaticæ, etc., the centrosome is of prime importance in spindle formation. There are, of course, cases which have not been thoroughly studied where this is not known to be the case. While this statement would hold true in general with animal cells, the writer is not sufficiently familiar with the literature to discuss the possible exceptions.

As to the third proposition there is a very great difference in different cases. The original idea of Boveri that the centrosome is a necessary and permanent *sui generis* organ of the cell, passing from cell to cell in division, has probably been abandoned by all investigators of the present day. It is claimed in various plants and animals to originate *de novo* in the cell, or at least become visible only at certain stages and in certain tissues. However, in all cases of genuine centrosomes known to the writer, they occur regularly in the cell divisions of certain tissues and seem to be mainly concerned with the spindle formation and division, having this as their prime if not sole and only function.

Considered in comparison with the above-described attributes of a centrosome, the blepharoplasts of *Zamia*, *Cycas*, and *Ginkgo* would seem to be very distinct organs. In *Zamia* the blepharoplast is located in the center of a very noteworthy aster, but when the spindle is formed there is found to be no connection between this and the blepharoplasts, which are located some distance outside the pole of the spindle. The same feature is very noticeable in *Cycas*, judging from Ikeno's figures 25a and 25b (70), and in *Ginkgo*, judging from Hirase's figures 18 and 19 (62).

The blepharoplast of *Zamia* has no discernible part in spindle formation, and it is certainly not a spindle-forming and division-directing organ. In no stage of the division have the spindle fibers any connection with it. The same can be said of *Cycas* and *Ginkgo*, so far as can be told by the investigations of Ikeno and Hirase. In *Ginkgo* in particular Hirase (62, fig. 18) figures an aster at the pole of the spindle inside of which a centrosome should be located, if present. The blepharoplast with its radiations, however, is located in the cytoplasm outside of this, the rays having apparently no connection.

In *Marsilia* the matter is more doubtful. Belajeff investigated the spermatogenesis of *Marsilia* particularly to determine the relationship of the blepharoplast to spindle formation, and he describes it as occurring at the pole of the spindle, thus fulfilling the requirement of position for a centrosome. Figures are given illustrating numerous spindle fibers extending from the nucleus to the blepharoplast, in the early stage of spindle formation, before the nuclear membrane has disappeared. It must be admitted that if these figures are directly translated in the light of previous knowledge of the centrosome question we can hardly escape the conclusion that the bodies must serve the purpose of a genuine centrosome in spindle formation, no matter what their later function may be. It seems surprising, however, that no radiations extend out from the blepharoplast into the cytoplasm on other sides than toward the nucleus when the spindle fibers would appear from the figures to be so plainly visible. The centrosome usually forms the center of an aster, the rays of which extend out in all directions. Yet judging from Belajeff's figures there is no indication of such radiations in *Marsilia*. It would seem possible that in *Marsilia* the blepharoplast may be independent of the spindle, though occupying a position near the meeting point of the converging spindle fibers. Such a body being present in the cell and normally in close proximity to the pole of the spindle, it is not surprising that it might appear in some instance to be nearly related to the spindle. Strasburger (112, p. 198) says that the blepharoplast is active kinoplasm, and that its collection at the pole of the spindle in spermatogenous cells of *Marsilia* does not signify particularly as to its relation to the spindle threads.

The writer is well aware that the great majority of investigators would consider Belajeff's figures and investigations as conclusive evidence of the centrosome nature of the blepharoplast, and the views of this brilliant investigator must meet with careful consideration. The matter is far from settled, however. In the light of Strasburger's investigations on swarm-spore formation and the origin of the cilia in these organs from a blepharoplast, the independent nature of the blepharoplast can not be set aside without further light on the spindle formation in *Marsilia*.

In regard to the occurrence of the blepharoplasts of *Zamia* it may be said that they are of very limited duration, occurring only in the central cell, where they originate *de novo*, and enduring through the division of this and the formation of the spermatozoids. They occur thus in only a single division with which they have no material connection. After fecundation they are lost and do not appear again until the central cells of the next generation are developed in the pollen grains. The same is true also of *Cycas* and *Ginkgo*. In *Marsilia*, according to Shaw (102), bodies similar to the blepharoplasts, which

he calls blepharoplastoids, occur in the division preceding that, giving rise to the central cell, and according to Belajeff (14) they appear a few cell generations earlier, but still in the spermatogenous tissue. It is easy to understand why blepharoplasts should occur in all of the spermatogenous cells resulting from the division of the central cell of the antheridium, as all of these cells may be considered potential spermatozoids. While in *Marsilia* 16 spermatids are formed by four successive divisions of the central cell, in some other species a less number of cells is formed, some of the intermediate divisions being dropped out. It seems to the writer, from analogy with *Zamia* and *Ginkgo*, where the blepharoplasts appear in the central cell, that they may be expected to occur also in the central cell of *Marsilia* and other ferns with which the central cell of the prothallum (antheridium) of *Zamia* is supposed to be homologous. It will be remembered that Moore found rudimentary cilia developing in the spermatozoa mother cells of salamander. All of the intervening cells between the central cell and the spermatids being considered as potential spermatids, it becomes evident that we should expect blepharoplasts or their rudiments to be present. The fact brought out by Shaw and Belajeff that these bodies apparently appear *de novo* in each cell generation and then at the close of the division disappear in the cytoplasm, new blepharoplasts arising meanwhile to function in the next cell generation, seems to the writer to lend strong support to his claim of the independent nature of the blepharoplast.

The evidence from the zoological standpoint would seem to entirely favor the centrosome nature of the blepharoplast, as the almost unanimous conclusion drawn in recent work on spermatogenesis indicates that the axial filament arises from a centrosome which forms the middle piece of the spermatozoid. After the exhaustive researches of Meves (82), Hermann (55), Moore (86), Benda, Lenhossek, Suzuki, McGregor, Paulmier, etc., this fact can hardly be doubted. Wilson says, in summarizing the questions of spermatogenesis in animals:

> In reviewing the foregoing facts we find, despite many variations in detail, three points of fundamental agreement, namely: (1) The origin of the sperm-nucleus from that of the spermatid; (2) the origin of a part at least of the "middle piece" from the spermatid-centrosomes; and (3) the outgrowth of the axial filament from one of the spermatid centrosomes.

Wilson (130, p. 170) also concluded, as stated above, that the cilia-forming organ in *Zamia*, *Cycas*, *Marsilia*, etc., is to be homologized with a centrosome, and the same conclusion is indicated in Henneguy's discussion of the matter (54). It would be presumptuous on the part of the writer to criticise these conclusions so far as they relate to the question of spermatogenesis in animals, and they must be accepted by him as they stand. He feels, however, that he is justified in refusing to admit, at least with the present evidence, that this must be taken

as settling the matter for plants also, where the whole centrosome question is on an entirely different plane.

In connection with Strasburger's theory that the blepharoplasts of plants are derived from the cilia-forming organs of asexual swarmspores, it is interesting to note that in ordinary ciliated animal cells a small, refractive, highly stainable body is developed at the base of each cilium with which it is connected—the so-called "basal knob" which lies near the periphery of the cell. These bodies have recently been considered by Henneguy (53) and Lenhossek (78) as of the same nature as the centrosome. A recent contribution to this question by Studnicka (113) is of special interest in this connection. He has studied the position and relation of this body to the cilia in numerous invertebrates and vertebrates in different ciliated cells, and concludes that this body ("*Fussstücke*" or "*Blepharoplast*") can not be surely identified with a centrosome. It is of special interest that in very many instances he found centrosomes near the blepharoplasts in the same cell (in *Salamandra maculata* and *Petromyzon fluviatilis*). It logically follows from this, as he states, that it "is hardly justified to always see in blepharoplasts specialized centrosomes." It would follow from this, if the basal knob can be considered a blepharoplast, that they can exist independently in a cell near the centrosomes, from which it follows that they can also appear in cells where no centrosomes exist.

In tracing the derivation of the blepharoplasts it may be argued that while the centrosome is not at present developed normally in the various tissues of the Cycads, *Ginkgo*, etc., at one time they were formed normally in the various tissues, and in the course of phylogenetic development have been gradually eliminated from the plant in general, being preserved and specialized only in the case of the spermatogenous cells where they serve an important and special function. No evidence has as yet been brought forward, however, on which such a conclusion can be based. Strasburger's recent researches (112) are of the greatest importance in pointing out the possible derivation of the blepharoplast from organs other than centrosomes. He takes the view that the blepharoplasts of *Zamia*, *Ginkgo*, etc., are homologous to the cilia-forming organs of swarmspores in lower plants, and have been derived from them. In the formation of the swarmspores in *Vaucheria*, *Edogonium*, *Cladophora*, etc., Strasburger has found that the nuclei approach the plasma membrane of the cell, toward which it becomes somewhat stretched out in the form of a beak. At the point where the nuclear beak or extension touches or approaches the plasma membrane a lens-shaped thickening of the membrane occurs from which the cilia are developed, a small knob being discernible at the base of each cilium. It has been thought that each of these knobs might represent a centrosome, but if so, they would be numerous and difficult to account for. No connection has been traced either between

these numerous basal knobs of the cilia or the entire lens-shaped body from which they develop and a centrosome existing in previous cell divisions. In the case of *Œdogonium*, furthermore, as pointed out by Strasburger, there would seem to be no centrosomes present, judging from the researches of Mitzkewitch, who figures the spindle as drawn to a point at the pole without a centrosome. Strasburger concludes that the lens-shaped thickenings on the plasma membrane from which the cilia develop in the case of *Vaucheria*, *Œdogonium*, etc., are to be considered the homologues of the blepharoplasts in *Zamia*, etc. He says:

> This organ we will at once designate as a blepharoplast, as I consider it homologous to the blepharoplasts of plant spermatogonia. The name was well selected; at least, I know of no reason for changing it.[1]

The development of sexually differentiated gametes is generally admitted to have taken place by development from swarmspores, and a study of plants showing early stages of sexual differentiation is thus of importance. Strasburger points out that in *Volvox*, which is such a plant, the cilia originate in a *Mundstelle* similar to that of *Œdogonium*, which he considers to be a blepharoplast. This derivation of the blepharoplasts of the *Cycadaceæ* from similar organs existing in lower plants is of the highest importance and indicates a general similarity in the mode of forming cilia in all motile reproductive cells.

Many important points yet remain to be determined in regard to the blepharoplasts before the controversy regarding their nature can be finally settled. No final conclusion can be reached, furthermore, until our knowledge of the typical centrosome has been extended and systematized so that it is possible to state what constitutes a centrosome. If the writer by his efforts has in any degree aided in paving the way to an earlier understanding of the matter, he is satisfied.

SUMMARY.

(1) The researches have shown that there are at least two species of *Zamia* in Florida, where only one has heretofore been recognized as occurring. These are *Zamia floridana* DC. and *Z. pumila* L. It was found that neither of the forms studied could be referred to *Z. integrifolia* Jacq., as has been done heretofore, this being a very distinct West Indian species.

(2) *Zamia* cones in various stages of development can be shipped by mail or express at least a three to five days' journey, and arrive in perfectly satisfactory condition for microscopic embryological study. Material preserved in the cone for six to ten days, as this requires, has been carefully compared with freshly cut material and found to

[1] Dieses Organ wollen wir gleich als Blepharoplasten bezeichnen da ich es für homolog den Blepharoplasten der pflanzlichen Spermatogonien halte. Der Name kann als gut gewählt gelten, zum Mindsten sehe ich keine Veranlassung ihn zu ändern.

have undergone no perceptible change. Zamia material in good condition for demonstration and for careful study of the details of spermatogenesis and fecundation can thus easily be obtained by any of the universities in Eastern United States. The comparative dates of development of different stages is given in the paper.

(3) Pollination is accomplished by the wind. The scales of the cone gradually reflex from the base upward in regular sequence, leaving an opening about one-fourth of an inch wide between the scales when fully open, into which the pollen must be blown to cause fecundation. When blown into the cone in this way it naturally rattles down to the axis of the cone near the micropyle of the ovary. In the further process of pollination a mucilaginous fluid is evidently extruded, which catches the pollen grains and is later drawn into the pollen chamber at the apex of the nucellus, either by absorption or by suction created by the breaking down of the tissue in the formation of the pollen chamber. In this way the pollen grains come to lie in the pollen chamber at the apex of the nucellus, where they germinate and form the spermatozoids.

(4) The mature pollen grain has two small prothallial cells cut off on one side of the grain, which are developed while the grain is still in the pollen sac. Indications of a resorbed prothallial cell have been observed in mature pollen grains and in grains shortly after germination. While the development of the pollen grain has not been followed, and the matter is somewhat doubtful, yet it is believed that three prothallial cells are cut off occasionally, if not regularly, the first of which is uniformly resorbed, as in the case of *Ginkgo* and *Pinus*. The two cells which remain plainly evident in the pollen-grain cells are referred to as the first and second prothallial cells, in the order of their formation.

(5) In the development of a stalk cell and central cell (generative cell or *Körper* cell) *Zamia* is found to correspond very closely to the Coniferæ as described by Strasburger and Belajeff. The general process is obscured, however, by the early development of the prothallial cells before the division of the second prothallial cell occurs. The first prothallial cell early begins to arch out into the second prothallial cell. As the development progresses this continues till the second prothallial cell comes to surround the main body of the first prothallial cell. The division of the second prothallial cell occurs after this condition is formed and the lower end of the spindle is crowded to one side by the intruding first prothallial cell. When the wall separating the stalk cell and the central cell is formed it is located near the apex of the first prothallial cell, so that the puzzling appearance of a cell surrounding a cell is formed. The nucleus of the stalk cell is always crowded to one side by the first prothallial cell. The writer has found the same process of development to occur in *Ginkgo* also.

(6) Shortly after the completion of this division the blepharoplasts

arise in the central cell, being formed *de novo* in the cytoplasm either in close proximity to the nuclear membrane or midway between the nuclear membrane and cell wall. They are at first very small, being scarcely more than points where a few radiating filaments converge. No distinct granules or differentiated central organ can be detected at this time.

(7) The blepharoplasts gradually increase in size, an outside surrounding membrane and vacuolated contents of different structure and composition being soon differentiated. They continue to grow until they reach a size, shortly before division, of about 18 to 20 μ in diameter. The kinoplasmic filaments, of which there were at first very few, increase in number until they become very numerous. The entire central cell and nucleus, together with the stalk cell and nucleus also, grow very materially in size.

(8) The prophase of division of the central cell appears to be the same as in ordinary cells. In this stage the blepharoplast has reached its largest size and has frequently become elliptical. Its contents present a beautiful, regularly vacuolate structure, and stain red with safranin.

(9) A synapsis stage is formed in the division of the central cell similar to the synapsis stage in the division of the pollen mother cells of various plants. This condition is not due to contraction, as the entire nucleus is filled with an unstained ground plasm which exhibits a reticular structure and shows no indication of contraction. In the collection of the chromatin matter around the nucleolus the chromatin granules apparently move along the meshes of this reticulum.

(10) As the division approaches the equatorial-plate stage the blepharoplasts begin to break up, the contents contracting and gradually disappearing, while the outer membrane begins to break apart here and there and can be observed to be made up of very numerous granules. The kinoplasmic filaments surrounding the blepharoplast, which in the previous stage had been very abundant, seem to have disappeared or at least are unrecognizable from the surrounding reticulum.

(11) The spindle is developed while the nuclear membrane is intact throughout, being apparently entirely of nuclear origin. In the equatorial-plate stage none of the spindle fibers can be traced beyond the nuclear membrane, and certainly have no connection with the blepharoplast.

(12) In an early anaphase the stainable contents of the blepharoplast have entirely disappeared, its place being taken by a colorless ground plasm. The outer membrane is more segmented and the individual granules of the membrane are clearly distinguishable. The nuclear membrane has broken down and its place is occupied by spindle fibers, which preserve the original shape of the nucleus. The spindle is fully formed now and the poles push very slightly out of the original nuclear limitations. There is no system of radiations surrounding the

pole of the spindle. In well-stained sections the spindle fibers can be seen to end abruptly in a loose reticular cytoplasm, a specialized area of which surrounds the pole of the spindle in the locations where the daughter nuclei are to be organized. The radiations surrounding the blepharoplast still exist in this stage, but are by no means so plain and abundant as in earlier stages. They have no connection with the spindle fibers, and end in the cytoplasm before they reach the specialized area of cytoplasm surrounding the spindle pole.

(13) In no stage have the kinoplasmic radiations of the blepharoplast been observed to grow in and take part in spindle formation, or in any way have any connection with the spindle other than that they lie in the line of symmetry of the cell, being naturally just outside the poles of the spindle.

(14) As development progresses the blepharoplasts break up entirely into numerous granules, the granules being apparently the same as those visible in the structure of the membrane of the blepharoplast in the last stage. By the time the blepharoplast has reached this stage the daughter nuclei have been fairly well organized.

(15) During the formation of the cell plate by the contraction and metamorphosis of the spindle fibers the process of organizing the cilia-bearing band from the blepharoplast is in progress. At first a slight line can be observed protruding slightly from the mass of granules of the blepharoplast. This line gradually increases in length, and one grows out on the opposite side of the mass of granules in the same way. Finally this line can be observed to have a definite width, which gradually increases. Careful observation shows this band to be formed by the fusion of the granules of the blepharoplast, a fact first pointed out by the writer. As the band continues to grow in length and width the blepharoplast granules gradually disappear until finally all are used up. The daughter nuclei by this time have reached a resting condition and form the spermatid cells which later become metamorphosed into the spermatozoids.

(16) A feature brought out in the writer's studies of *Zamia* for the first time is that here the entire spermatid cell is metamorphosed into a spermatozoid, there being no differentiation of spermatozoids inside of a mother cell, as was previously understood to be the case in the spermatogenesis of plants.

(17) The band formed, as above described, continues to grow in length some time after the disappearance of the granules of the blepharoplast. At this time it has usually formed one turn around the spermatid. It is first located in the cytoplasm midway between the nucleus and periphery of the cell, but ultimately moves out and becomes appressed against the plasma membrane. It assumes the form of a helicoid spiral as it elongates and finally makes from five to six turns around the cell. In a very early stage protuberances can

be distinguished on the outer surface of the band which ultimately grow into cilia.

(18) While the growth and division of the central cell has been taking place tissue changes have occurred in the upper part of the nucellus and the pollen-grain ends of the pollen tubes have grown down so that they hang free into the archegonial chamber over the neck cells of the archegonia. In fecundation the pollen tubes grow down until they crowd against the neck cells, and, being under severe tension, burst and discharge the spermatozoids over the archegonia. The fluid for the swimming of the spermatozoids is surely formed in part from the pollen tube and may be partially formed by extrusion from the egg cell.

(19) The mature spermatozoids are the largest known to occur in any plant or animal, being visible to the unaided eye. Their motions have been carefully studied by keeping them alive in sugar solutions. They are ovate or nearly spherical, the apex of the ciliferous spiral being usually more or less pointed. Their motion is mainly by the aid of the cilia, but besides this they have a sort of selective amœboid motion of the spiral end.

(20) In fecundation the entire spermatozoid enters the egg cell, swimming in between the ruptured neck cells. Sometimes two or three spermatozoids enter the same egg, but only one is used in fecundation, the others perishing.

(21) On entering the upper part of the egg cytoplasm the nucleus escapes from the spermatozoid, being left slightly in rear of the active ciliferous band. The plasma membrane of the spermatozoid entirely disappears, seeming to unite with the cytoplasm of the egg, and this allows the spermatozoid cytoplasm also to unite with the egg cytoplasm and leaves the nucleus free. The ciliferous band remains at the apex of the egg cell in the cytoplasm and the nucleus passes on to the egg nucleus, with which it unites.

(22) Fecundation thus consists of a fusion of two entire cells—cytoplasm with cytoplasm and nucleus with nucleus.

(23) The first division of the egg nucleus has not been observed, but the second and later divisions have been carefully studied. In no case of the cleavage divisions has any centrosome been observed or other body at the pole of the spindle which might be confused with a centrosome. The development has been followed until the embryo is fairly well organized, so that it may be concluded that there is no centrosome present in the divisions closely following the first cleavage of the egg nucleus. It is certain that the ciliferous band, which represents the blepharoplast of the spermatid, has no function in the formation of the first cleavage spindle or the spindles in any of the divisions immediately following, as it remains intact at the apex of the egg cell until the egg nucleus has divided into very many small nuclei.

It disappears later, during the formation of the embryo, being apparently absorbed during the process.

(24) The function of the blepharoplast from the results obtained in this study appears to be simply the formation of the motile cilia and the transportation of the male cell. It forms the machinery of locomotion.

(25) The greatest interest in the present paper is in the relation of the blepharoplast to centrosomes or centrospheres. They are found to differ from centrosomes as generally understood (1) in not forming the center of an aster at the pole of the spindle, being located entirely outside of the spindle in *Zamia*, *Ginkgo*, and *Cycas;* (2) in having no connection with spindle formation; (3) in being limited to the division of a single cell, thus to one cell generation, no similar organ appearing in any other stage of the plant's development, so far as known, and (4) in having a function differing from that of any typical centrosome, so far as known in plants.

(26) Considering the organs distinct from centrosomes, the writer in an earlier preliminary paper called them *blepharoplasts.* This the writer contends was justifiable and proper, even if the organs are finally proven to be the homologues of centrosomes. They are now very certainly specialized organs functioning only as cilia formers.

WASHINGTON, D. C., *May 1, 1901.*

NOTE.—Since this monograph went to press several important papers bearing directly on the subject have appeared, but as these do not serve to change the writer's conclusions or materially affect the discussion, no special consideration of them is here necessary. The most important of these is Dr. S. Ikeno's paper entitled "Contribution a l'étude de la fécondation chez le Ginkgo biloba," published in 1891 (Ann. d. Sci. Nat. Bot. VII, sr. 13: 305–318).

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EXPLANATION OF ILLUSTRATIONS.

All of the figures were drawn with the aid of a camera lucida, and where high magnification was used, with Zeiss apochromatic objectives 3 mm., n. a. 1.40, or 2 mm., n. a. 1.30.

PLATE I.

Zamia floridana.

Fig. 1. Median longitudinal section through upper part of ovule at time of pollination: *c*, coat of ovule; *m*, micropyle; *pc*, pollen chamber; *n*, nucellus; *p*, prothallus. (× 18 diam.)

Fig. 2. Median cross section of female cone at time of pollination. (½ nat. size.)

Fig. 3. Mature pollen cone with pollen shedding. (½ nat. size.)

Fig. 4. Cross section of mature pollen cone, showing pollen sacks on lower surface of scales. (½ nat. size.)

Fig. 5. Median longitudinal section through apex of nucellus, showing pollen chamber and developing pollen tubes. (× 100 diam.)

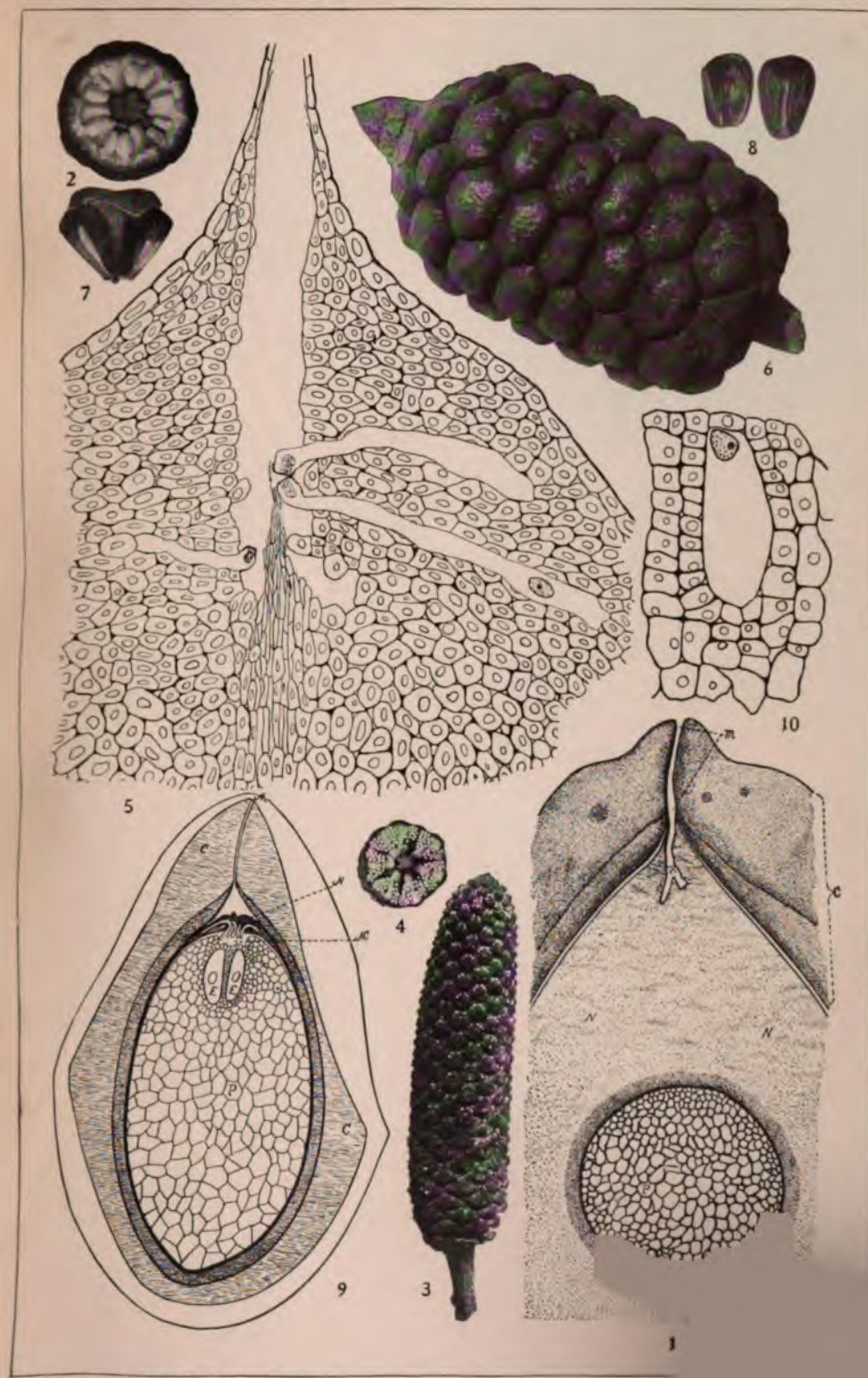
Fig. 6. Cone at time of fecundation, having reached maximum size. (½ nat. size.)

Fig. 7. Scale and attached seeds at time of fecundation. (½ nat. size.)

Fig. 8. Two seeds at time of fecundation, having reached maximum size. (½ nat. size.)

Fig. 9. Median section through seed just before fecundation, showing relative size and location of parts (diagrammatic); *c*, coat of ovule; *m*, micropyle; *n*, nucellus, showing pollen tubes hanging down into the archegonal chamber *ac; e*, egg cell; *p*, prothallus. (× 3 diam.)

Fig. 10. Median section through young archegonium, showing central cell and nucleus before the cutting off of the canal cell. (× 100 diam.)



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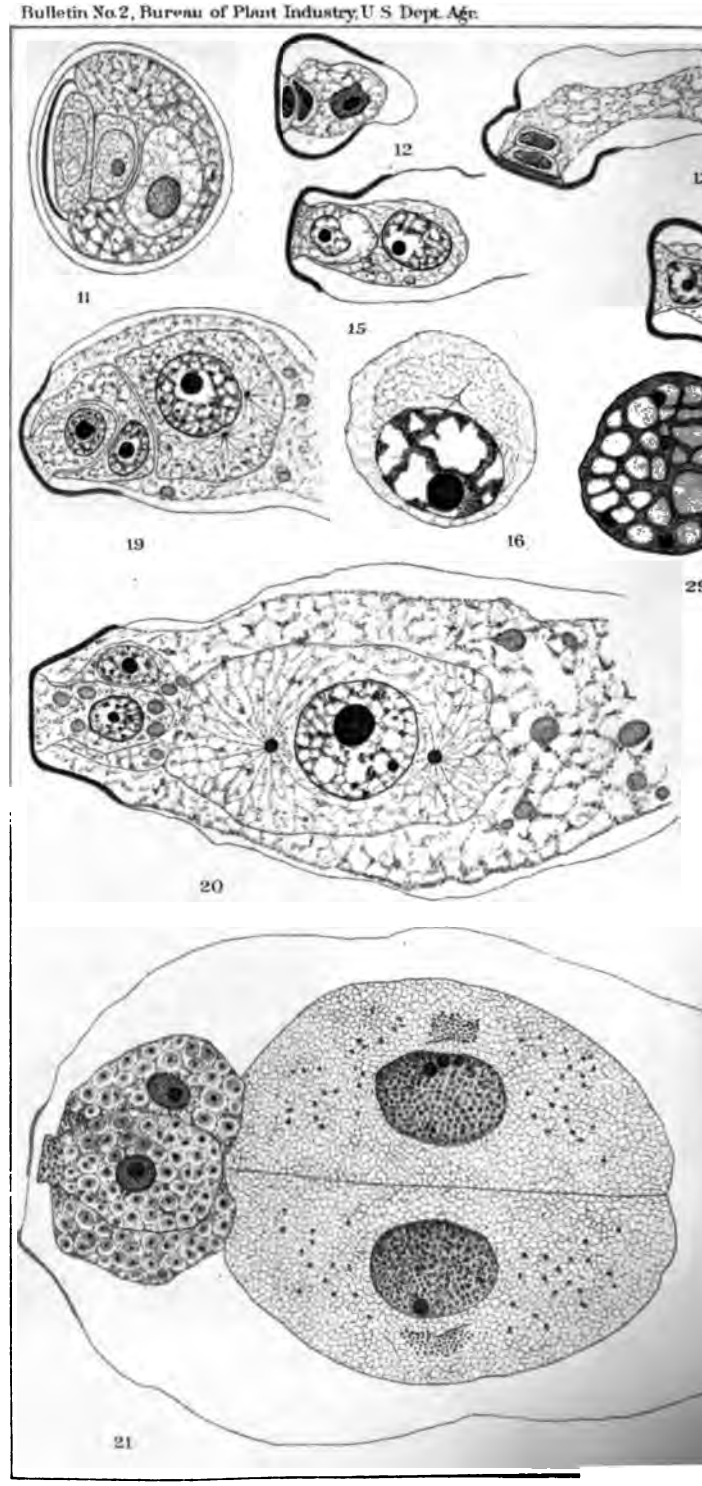
SPERMATOGENESIS OF ZAMIA FLORIDANA.



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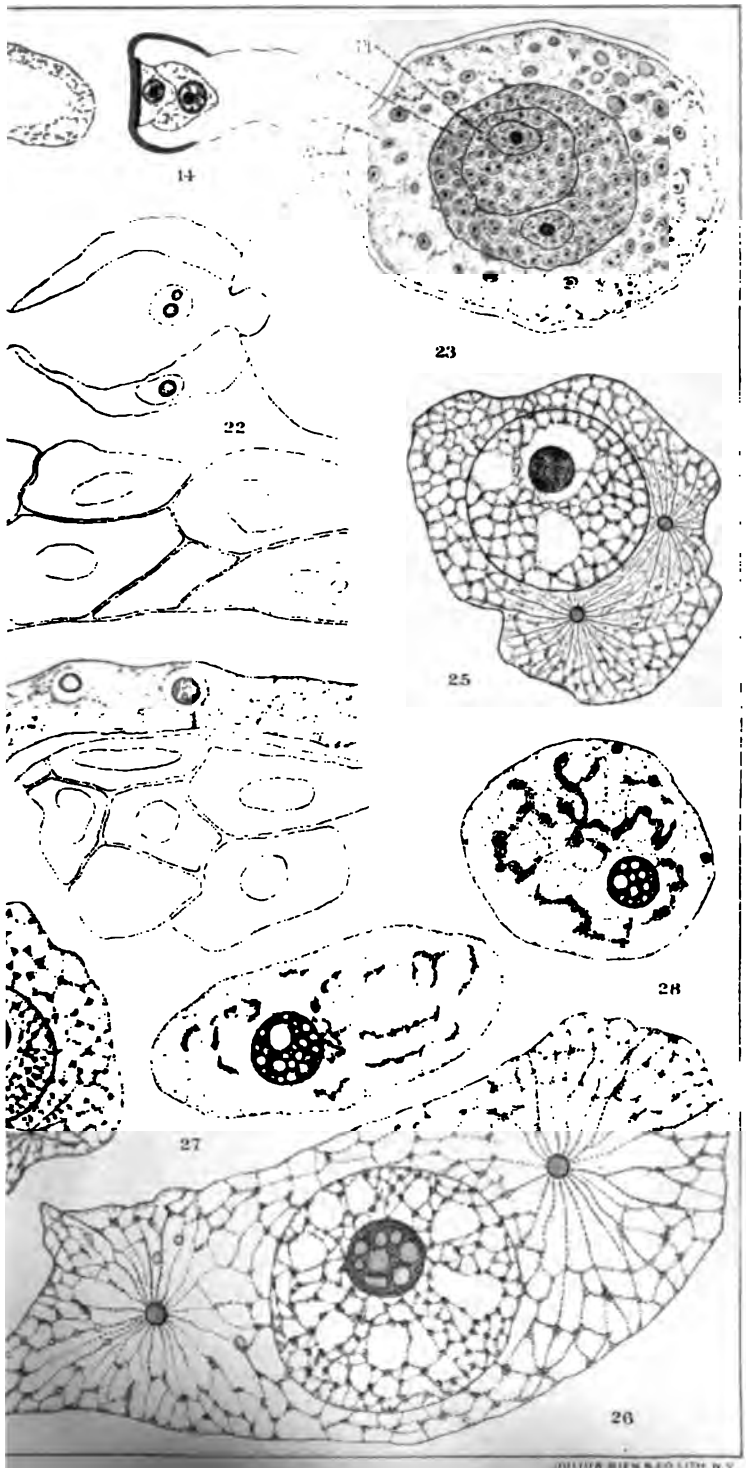
SPERMATOGEN

PLATE II



DANA AND ZAMIA PUMILA

PLATE II.

Zamia floridana and *Zamia pumila.*

Fig. 11. Mature pollen grain in water. At the point of attachment of the two prothallial cells, on the left, a dark crescent-shaped line represents a dark layer in the cell wall of pollen grain, which may be the remains of a third resorbed prothallial cell. (× 1,200 diam.)

Fig. 12. Germinating pollen grain, early stage. The two prothallial cells have not yet begun to increase in size. (× 600 diam.)

Fig. 13. Germinating pollen grain, later stage. The tube nucleus has increased in size and has passed out of the old grain into the tube, the prothallial cells still remain unchanged. A dark line at base of attachment of prothallus in this tube may be the remains of a third prothallial cell. (× 600 diam.)

Fig. 14. Germinating pollen grain, later stage. Here the first prothallial cell has just started to push out into the second prothallial cell. (× 600 diam.)

Fig. 15. Germinating pollen grain, later stage. The first prothallial cell has crowded out into the second prothallial cell in a marked degree. (× 600 diam.)

Fig. 16. Transverse section of second prothallial cell in early prophase of division, showing one of the bodies which may occasionally be found, that resemble the early stages of a blepharoplast. (× 1,200 diam.)

Fig. 17. Pollen tube penetrating nucellar tissue, and showing the nucleus of the second prothallial cell in division, the lower end of the spindle being crowded to one side by the intruding first prothallial cell. (× 600 diam.)

Fig. 18. Prothallus of pollen tube immediately after the completion of the division of the second prothallial cell into a stalk cell and central cell. Two starch grains are shown here in the second prothallial cell. (× 600 diam.)

Fig. 19. Prothallus of pollen tube in a later stage of development after the appearance of the blepharoplasts. The double plasma membrane separating the first prothallial cell and stalk cell, which is here visible, shows that there are two distinct and independent cells of separate origin. (× 600 diam.)

Fig. 20. Prothallus of pollen tube in a later stage of development after central cell has become elongated and the blepharoplasts have taken position on opposite sides of the nucleus, corresponding to the longitudinal axis of the pollen tube. Starch grains have begun to appear in the stalk cell, etc. (× 600 diam.)

Fig. 21. Prothallus of pollen tube very much later after the division of the central cell; the blepharoplasts have separated into granules which are starting to organize the ciliferous band. The first prothallial cell and stalk cell have become gorged with starch. The original size of the attachment of the first prothallial cell is clearly shown at base of that cell. (× 300 diam.) This tube is magnified only one-half as much as that shown in fig. 20.

Fig. 22. Prothallus showing the interior first prothallial cell crowding into the central cell. (× 300 diam.)

Fig. 23. Cross section of pollen tube extending through stalk cell and interior first prothallial cell: *w*, wall of pollen tube; *cp*, cytoplasm of pollen tube; *sc*, stalk cell; P1, first prothallial cell. (× 300 diam.)

Fig. 24. Central cell with blepharoplasts shortly after origin. (× 1,200 diam.)

Fig. 25. Central cell with blepharoplasts near together and somewhat older, when outer membrane has been differentiated. (× 1,200 diam.)

Fig. 26. Central cell with blepharoplasts in median stage of development, showing relation of kinoplasmic radiations to reticulum of cytoplasm. (× 1,200 diam.)

Fig. 27. Nucleus of central cell in early prophase of division, the chromatin matter beginning to collect in granular masses. (× 350 diam.)

Fig. 28. Nucleus of central cell in later prophase of division, the chromatin having collected in a skein. (× 350 diam.)

Fig. 29. Blepharoplasts, showing vacuolated contents and refractive bodies resembling crystalloids. (× 1,200 diam.)

PLATE III.

Zamia floridana and *Zamia pumila.*

Fig. 30. Division of central cell, synapsis stage, showing the collection of the chromatin matter around the nucleolus, and reticular ground plasm filling the remaining portion of the nucleus. (× 350 diam.)

Fig. 31. Division of central cell, equatorial plate stage, showing the blunt-poled intranuclear spindle, the outer membrane of the blepharoplasts breaking up, and the contracting of the contents of the blepharoplast. (× 350 diam.)

Fig. 32. One of the blepharoplasts from the above cell more highly magnified, showing the breaking up of the exterior membrane and the disappearance of the contents. (× 1,200 diam.)

Fig. 33. Division of central cell, early anaphase, showing hyaline cytoplasmic areas around the poles and disconnection of the blepharoplasts with the spindle. (× 350 diam.)

Fig. 34. One of the blepharoplasts and the pole of the spindle from the above cell more highly magnified, to show the relation of the kinoplasmic rays surrounding the blepharoplasts to the spindle fibers, the granular structure of the outer membrane of the blepharoplast, and its separation and contents at this time. (× 1,200 diam.)

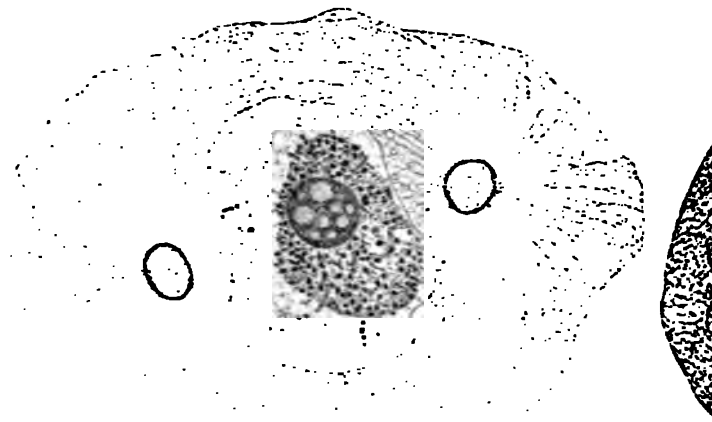
Fig. 35. Division of central cell, early telophase, showing the reorganization of the daughter nuclei. The blepharoplasts have separated into groups of granules, which in this stage, are nearly as large as the daughter nuclei. (× 350 diam.) (Compare this with a photomicrograph of the same cell, Pl. V, fig. 63.)

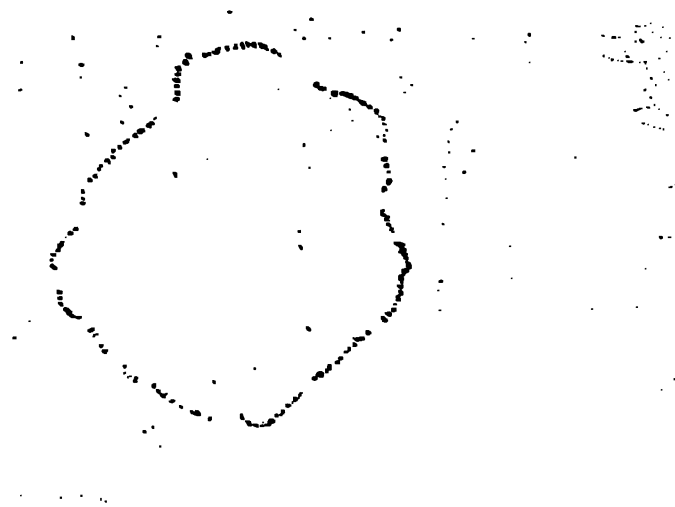
Fig. 36. Two attached spermatids formed by the completion of the division of the central cell. The blepharoplast is in the process of organizing the ci[illegible] band by the fusion of the granules. (× 350 diam.)

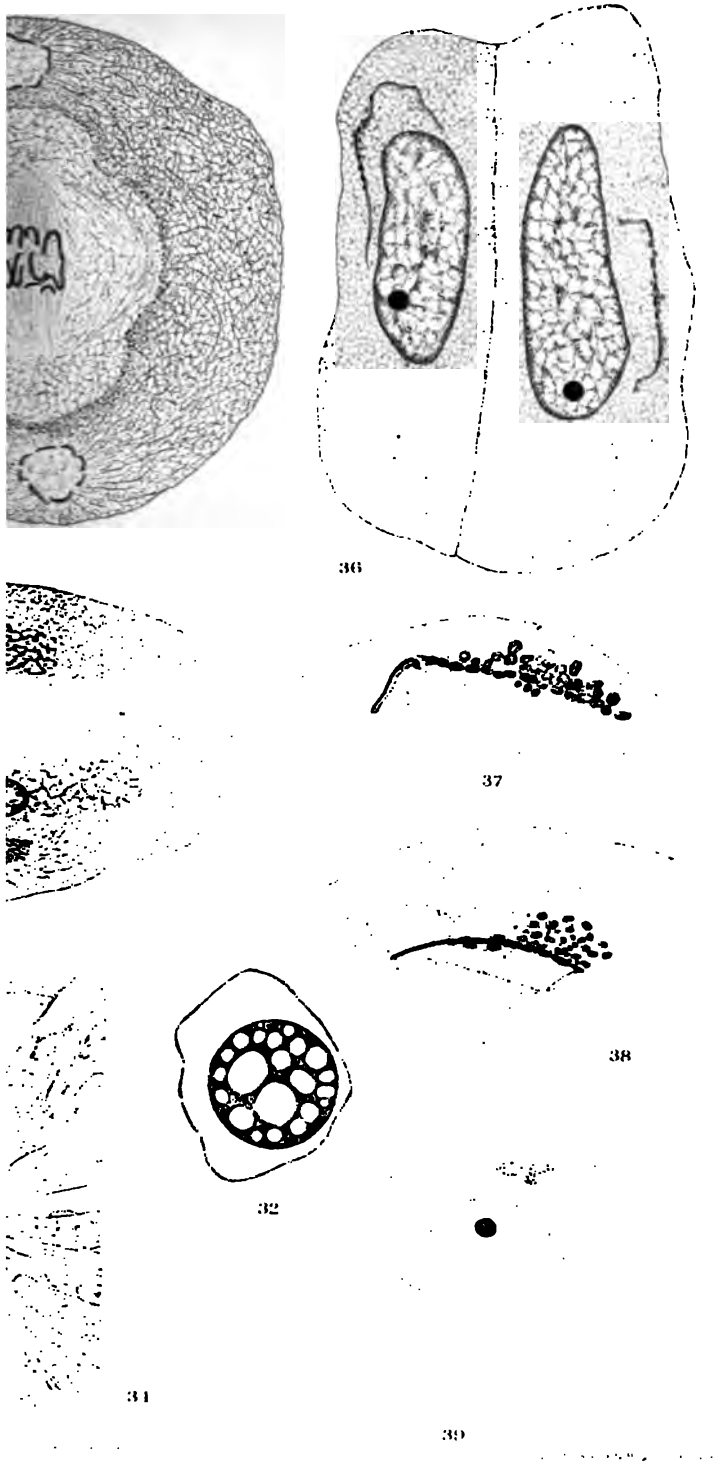
Fig. 37. Organization of the ciliferous band by a fusion of the [illegible]ules of the blepharoplast. (× 1,200 diam.)

Fig. 38. Fusion of the granules of the blepharoplast in the formation of the ciliferous band. (× 1,200 diam.)

Fig. 39. Spermatid showing irregular projections from the nucleus, and with ciliferous band in process of construction. (× 350 diam.)







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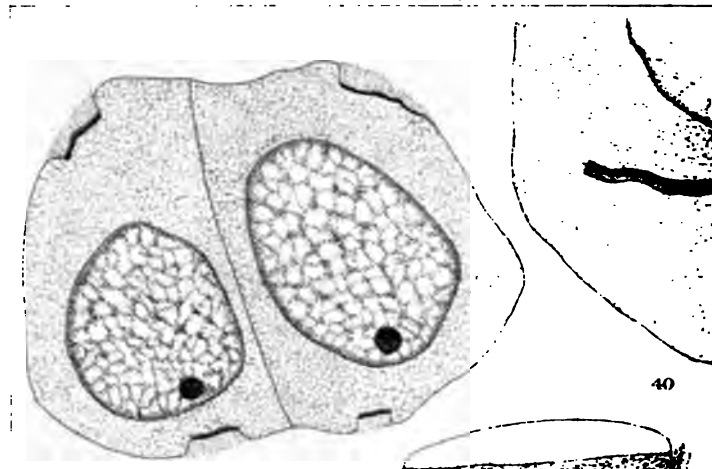
ANA AND ZAMIA PUMILA



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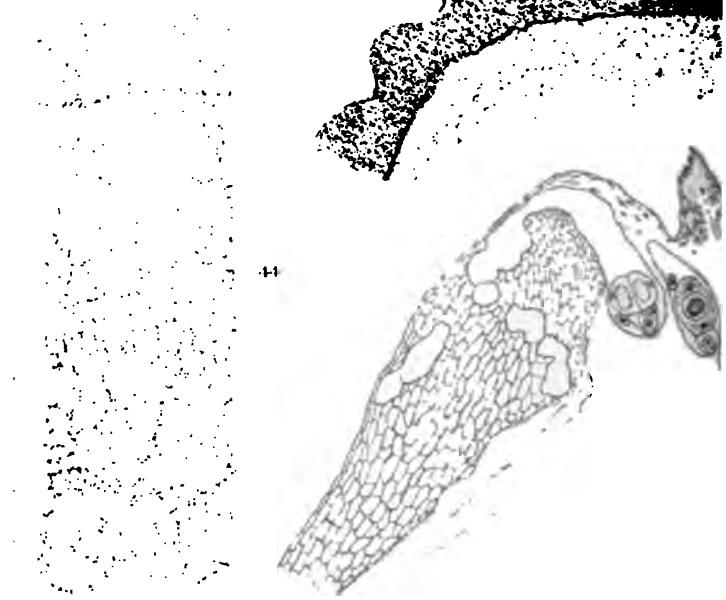

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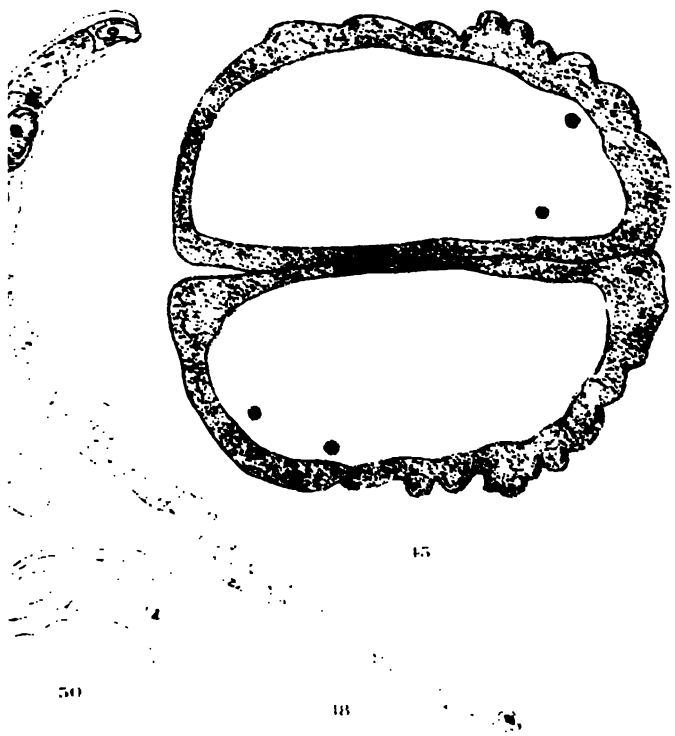

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PLATE IV.

Zamia floridana and *Zamia pumila.*

Fig. 40. Spermatid with section of ciliferous band in cytoplasm, showing protuberances on outer side. (× 600 diam.)

Fig. 41. Spermatid with section of ciliferous band in cytoplasm, showing radiations from outer surface. (× 350 diam.)

Fig. 42. Median section of two spermatids where ciliferous band has made a single turn around the cell, showing band in section appressed against the plasma membrane on opposite sides of the nucleus. (× 350 diam.)

Fig. 43. Tangential surface section of a spermatid, showing surface of band when it had made a single turn. (× 350 diam.)

Fig. 44. Formation of double plasma membrane in the division of the central cell. (× 1,800 diam.)

Fig. 45. Mature spermatozoids in median section, showing nuclei, ciliferous band, etc. (× 200 diam.)

Fig. 46. Cross-section of apex of spermatozoid, showing attachment of cilia to band which lies immediately below the plasma membrane. (× 600 diam.)

Fig. 47. Separation of spermatozoids under the influence of sugar solution: *a*, mature pollen tube just before motion began; *b*, *c*, and *d*, after the motion of the cilia had begun, showing stages in the gradual pulling apart of the spermatozoids.

Fig. 48. Pollen tube in a median stage of growth, showing the tube nucleus near the distal end of the tube. (× 75 diam.)

Figs. 49 and 50. Pollen tubes, showing different shapes assumed by the tubes in the course of the growth of the proximal end, just preceding fecundation. (× 75 diam.)

Fig. 51. Apex of nucellus, showing the proximal ends of the pollen tubes hanging down in the archegonial chamber shortly before fecundation. The tube nuclei have returned and taken position near the prothallus. (× 75 diam.)

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PLATE V.

Zamia floridana and *Zamia pumila.*

Fig. 52. Mature spermatozoid while swimming free. (× 200 diam.)

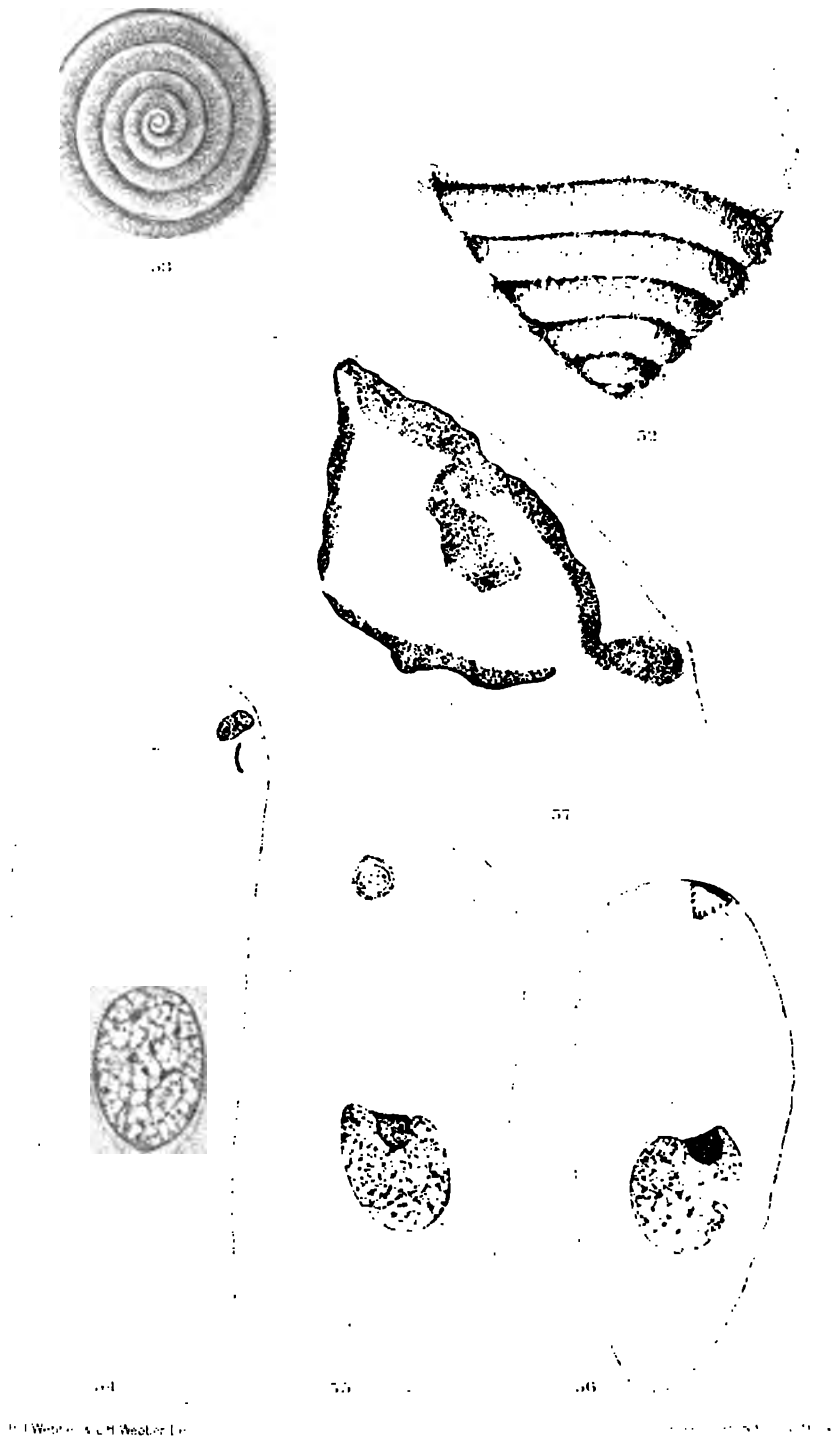
Fig. 53. Spermatozoid showing apex of spiral.

Fig. 54. Fecundated egg cell immediately before nuclear fusion, the nucleus of the spermatozoid having separated from the ciliferous band and cytoplasm, lies free in the protoplasm at the apex of the egg cell ready to travel on alone and fuse with the egg nucleus. (× 25 diam.)

Fig. 55. Egg cell immediately after the fusion of the male and female nuclei, showing male nucleus in the upper portion of the oosphere, and the isolated ciliferous band of the spermatozoid which produced the fecundation lying free in the cytoplasm at the apex of the egg cell. A second spermatozoid trying to gain entrance is shown at apex of cell. (× 25 diam.)

Fig. 56. Egg cell immediately after fusion of male and female nuclei as in fig. 55, showing longitudinal section of ciliferous spiral band and a portion of the cytoplasm of the spermatozoid. (× 25 diam.)

Fig. 57. Section through the apex of a fecundated egg cell showing the remains of the cytoplasm and ciliferous band of the spermatozoid surrounded by the cytoplasm of the egg cell. (× 350 diam.)



SPERMATOGENESIS AND FECUNDATION OF ZAMIA FLORIDANA AND ZAMIA PUMILA

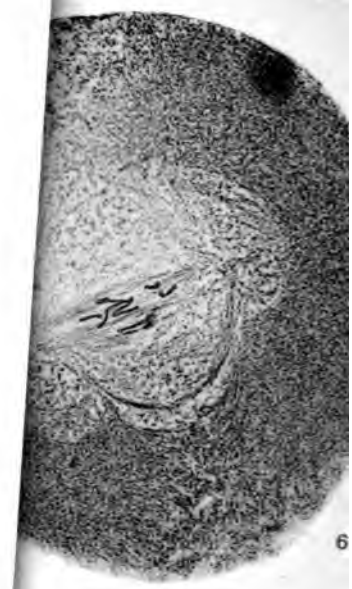


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PLATE VI.

Zamia floridana and *Zamia pumila*. Photomicrographs of various stages.

Fig. 58. Central cell in pollen tube showing blepharoplasts, nucleus, nucleolus, and tube nucleus below to the left. (× 200 diam.)

Fig. 59. Central cell in early prophase of division, showing blepharoplasts, contraction of nuclear membrane between blepharoplasts, nucleolus, and granular collections of chromatin matter. (× 300 diam.)

Fig. 60. Polar view of mature blepharoplast, showing radiations. (x 400 diam.)

Fig. 61. Blepharoplast, showing contraction of contents and breaking of outer membrane into segments. (× 400 diam.)

Fig. 62. Central cell in early anaphase of division, showing the relation of the blepharoplast (one only being visible) to the spindle. The clear, specialized cytoplasmic areas between the blepharoplast and the poles of the spindle are well shown. (× 400 diam.)

Fig. 63. Central cell in telophase of division, showing the blepharoplast in granules, the small daughter nuclei, and the formation of the cell plate by the contraction of the spindle fibers. (× 500 diam.)

Fig. 64. Polar view of the blepharoplast in slightly later stage, showing the fusion of the granules to form the ciliferous band. (× 400 diam.)

Fig. 65. Two spermatids where the ciliferous band has made one turn around the cell. (× 200 diam.)

PLATE VII.

Photomicrographs of *Zamia floridana*.

Fig. 66. Central cell in late telophase with the blepharoplast in granules and the delimiting plasma membrane well formed. (× 550 diam.)

Fig. 67. Mature spermatozoids in median section, showing nucleus, etc. (× 150 diam.)

Fig. 68. Mature spermatozoid swimming free in pollen tube. (× 200 diam.)

Fig. 69. Median section of helicoid ciliferous band in cytoplasm at apex of egg cell after escape of the nucleus, showing cilia still attached. Numerous little comet-like figures of kinoplasm formed here and there in the cytoplasm of the egg cell at this time are also shown. (× 450 diam.)

Fig. 70. Egg cell after fecundation, showing a spermatozoid which did not gain entrance and an empty ciliferous band in apex of egg cell. The egg nucleus is visible in the lower part of the section, but the male nucleus which has fused with it was not visible. (× 50 diam.)

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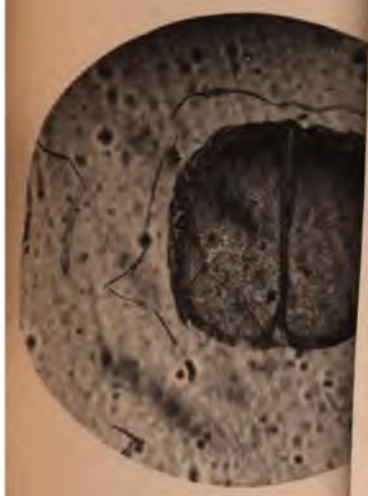

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN No. 2.

B. T. GALLOWAY, Chief of Bureau.

SPERMATOGENESIS AND FECUNDATION OF ZAMIA.

BY

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[1] Detailed to tobacco investigations, Bureau of Soils.
[2] Detailed to Seed and Plant Introduction.

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The Bureau of Plant Industry, which was organized July 1, 1901, includes Vegetable Pathological and Physiological Investigations, Botanical Investigations and Experiments, Grass and Forage Plant Investigations, Pomological Investigations, and Gardens and Grounds, all of which were formerly independent divisions, and also Seed and Plant Introduction, The Arlington Experimental Farm, Tea Investigations and Experiments, and the Congressional Seed Distribution. Beginning with the date of the organization of the Bureau, the independent series of bulletins of each division was discontinued and all are now published as one series of the Bureau.

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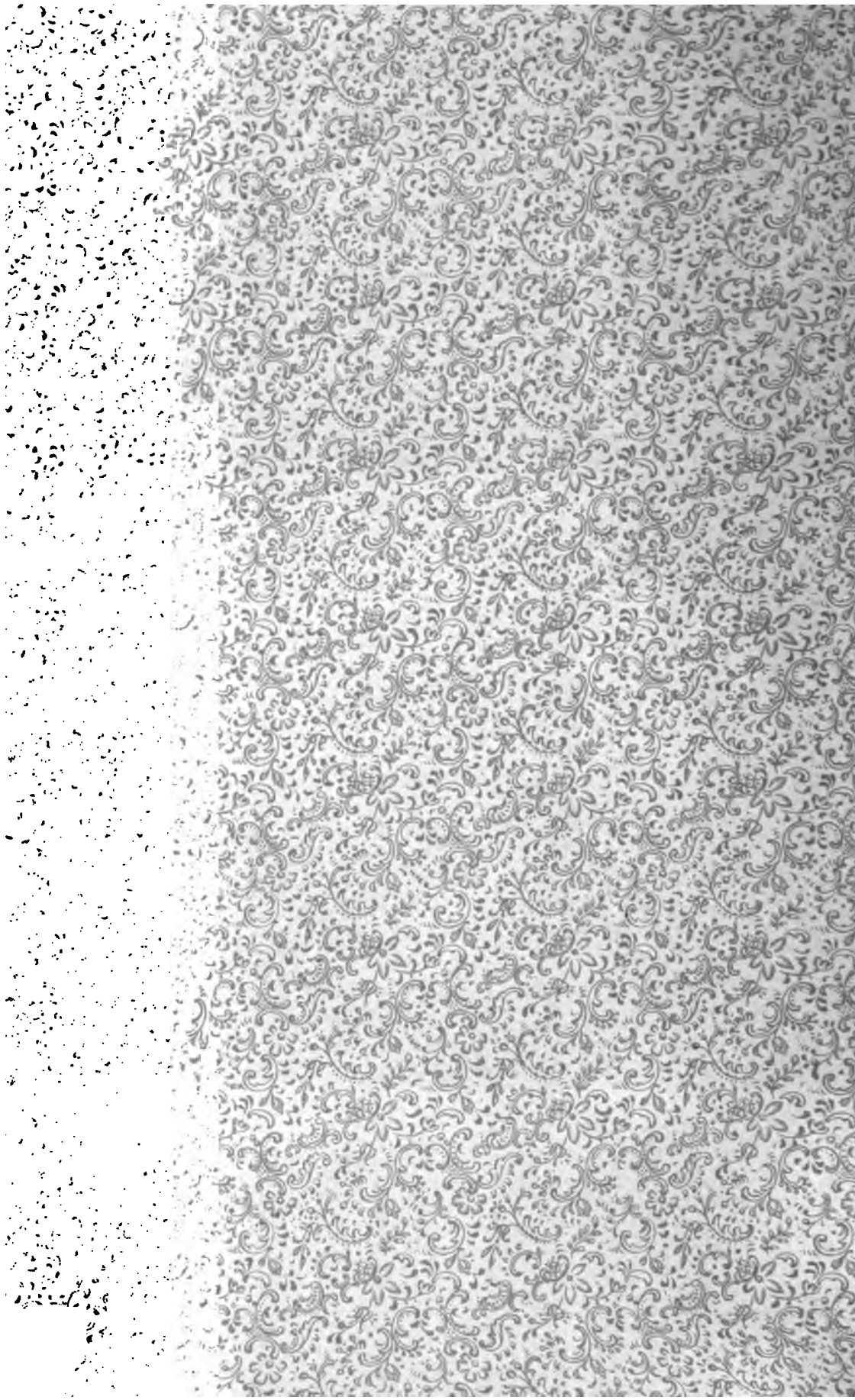

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